# *The*
# MICROSCOPE
## A Practical Handbook

*By*

AUBREY H. DREW, D.Sc., F.R.M.S.
AND
LEWIS WRIGHT

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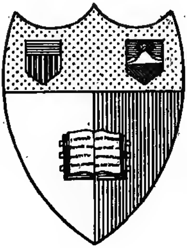

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# THE MICROSCOPE

## A PRACTICAL HANDBOOK

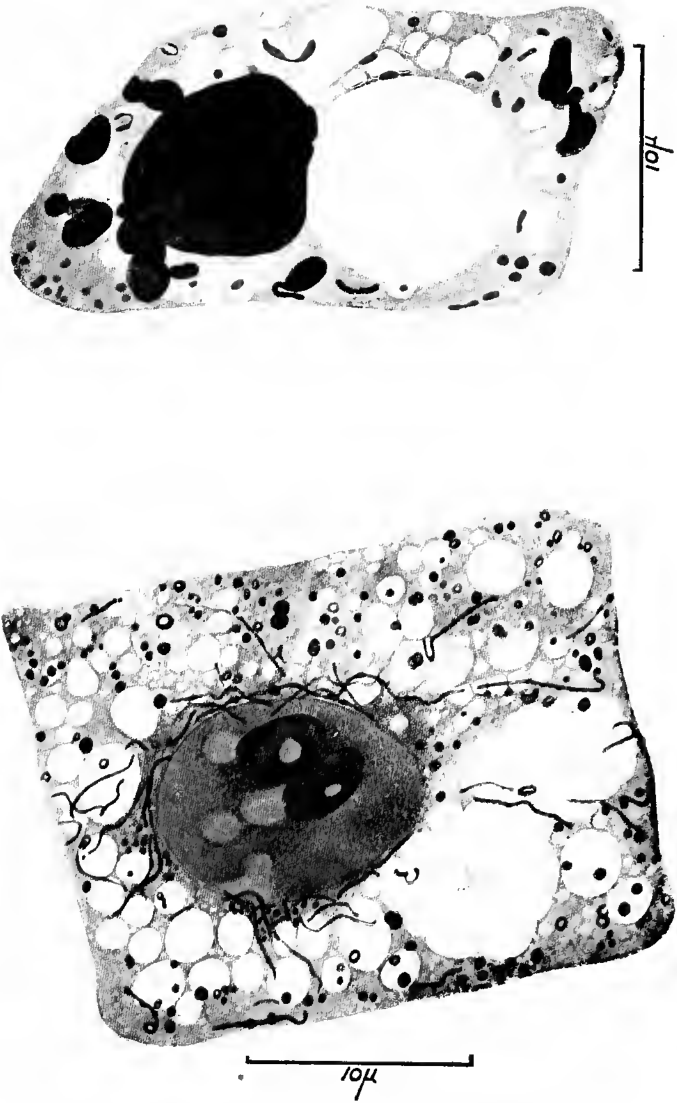

*Fig. 73.* Section of Onion Root showing the Mitochondria and Golgi Apparatus. This object forms an excellent test for lenses of medium and high power. Watson's 2 m.m. Apochromatic Oil Immersion Objective N.A. 1.37.

# THE MICROSCOPE

## A PRACTICAL HANDBOOK

BY

LEWIS WRIGHT

ENLARGED AND REWRITTEN

BY

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CORNELL

"But the greatest error of all is, mistaking the ultimate end of knowledge; for some men covet knowledge out of a natural curiosity and inquisitive temper, some to entertain the mind with variety and delight, some for ornament and reputation, some for victory and contention; many for lucre and a livelihood, and but few for employing the Divine gift of reason to the use and benefit of mankind."

—LORD BACON.

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# INTRODUCTION

THIS handbook, written many years ago by the late Mr. Lewis Wright, has proved so extremely useful to both amateurs and students that it has been decided by the publishers to have it rewritten and brought up to date. During recent years microscopic science has made remarkable progress, and in consequence thereof a very large portion of the work had become quite out of date.

As far as has been possible in re-writing the work I have endeavoured to follow the arrangement of Mr. Wright. In the chapters on apparatus I have adhered to the original plan, viz. to show the reader exactly what he can obtain for a given expenditure. It must be borne in mind, however, that since the war microscopes have increased in price very greatly, and that although recently there has been a tendency for prices to fall it seems unlikely that English instruments will ever be as cheap as formerly. Every stand referred to is known personally to me, and I have mentioned no lenses that I have not examined many times, so that the list given will be found perfectly trustworthy.

With regard to the selection of instruments, I have assumed that the reader has a real desire to study and learn something of the marvellous world revealed by the microscope. With this object in view I have endeavoured to guide the student to select an instrument with which real work may be done, and which he will not need to discard at a later date owing to its shortcomings. In describing manipulation, I have endeavoured to show the student in as simple language as possible how to use his microscope to the best advantage.

The later chapters, dealing with some of the revelations of the microscope, have undergone considerable change from the original form. I have purposely devoted more space in this section to the Protozoa and allied forms, as there is much useful work to be done here by the amateur, and work moreover which apart from the microscope does not need any costly appliances. With the view to assisting the student as far as possible to identify his specimens, several plates have been introduced in which the various organisms have been figured by means of line drawings.

Considerable space has been given to the consideration of the best methods of obtaining critical images, as this subject is still far from being understood either by Professors of histology or their students. It cannot be too strongly insisted that the microscope is not a mere magnifying glass, and can only be used as such, with an immense loss of resolution. There is probably no other scientific instrument which is put into the hands of the student without a detailed explanation as to its use; yet how often is instruction regarding the microscope given the students by their teachers! Indeed the Professors are in many cases as ignorant of the proper use of the instrument as are their students. Only recently, at a meeting where many microscopic objects were being shown, the writer heard one teacher of pathology remark, "I cannot understand all this talk of critical images," and yet this man was engaged in teaching students subjects requiring the constant use of the microscope.

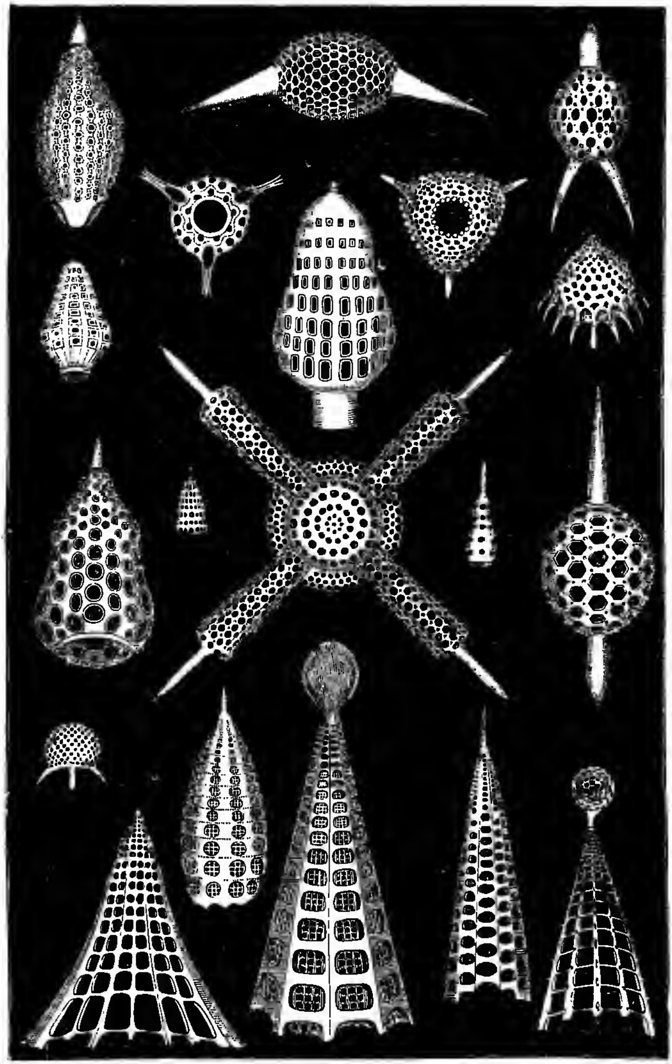
The chapter on section cutting and mounting has been considerably enlarged, so as to enable the student to gain some knowledge in the preparation of his own specimens.

It has been my aim in rewriting this work to extend its usefulness, and to provide an elementary text-book, which will prove of assistance, not only to the amateur, but to the medical and biological student, in seeking to learn the proper use of the microscope, which is essentially an instrument of the utmost precision.

I have to thank Messrs. Baker, Beck, Leitz, Ogilvy, Watson, Swift and Zeiss for the loan of numerous blocks, and also Dr. J. A. Murray and Mr. W. Pilgrim for kindly making a number of drawings.

A. H. DREW.

IMPERIAL CANCER RESEARCH FUND,
LONDON, 1922.



*Mungo Ponton, del*

FIG. 80*a*.—FOSSIL RADIOLARIA (POLYCYSTINA) × 100

# THE MICROSCOPE
# A PRACTICAL HANDBOOK

## CHAPTER I

### RAYS, IMAGES, LENSES

For the proper selection and intelligent use of a microscope and its accessories, some comprehension of the fundamental principles upon which the formation of images is based is essential.

**Images and Magnification.**—The physical act of 'seeing' results from an 'image' or picture of an object being thrown upon the retina or sensitive screen of nerve endings lying behind the crystalline lens of the eye. The apparent size of an object depends upon this image which is formed on the retina, and to magnify an image is to increase the visual angle formed on the retina (fig. 1). The eye itself is similar in construction to a camera, having a lens in front (crystalline lens and cornea) and a sensitive receiving screen (retina) behind.

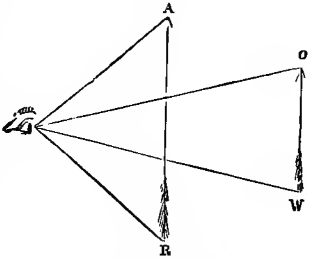


FIG. 1.—Visual Angle

Images are formed by the lens in front of the eye focussing the waves of light proceeding from the object on to the retina. The angle formed by the extreme rays of the object causes us mentally to form an impression of the size and distance of the object. For visual purposes of seeing an object closely the unit of distance employed is 10 inches. In magnification the eye mentally refers back all objects to this distance, and thus if an object is examined with a lens which permits it to be seen clearly at a distance of 1 inch, the angle so formed is ten times as great. The brain mentally refers back light along this angle to a distance of 10 inches, and the object appears correspondingly magnified.

**Images formed by Rays.**—In the last paragraph reference has been made to images formed by rays of light being focussed by the lens of the eye as in a camera. To appreciate this it must be understood that each ray of light is capable of forming an image, and this function is utilised by lenses. A single experiment will prove this. Light a candle in a dark room and support a sheet of card or white paper two feet away. Take also a large blackened card and prick a pinhole in the middle, holding this midway between candle and white card. By this means rays will be separated from the source of light, and it will be seen they form a real image or picture of the light source upon the card.

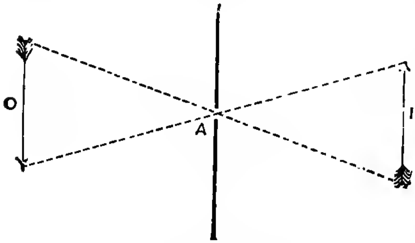


FIG. 2.—Inverted Image

**Inversion and size of Image.**—The image formed in this manner is inverted, as is evident from fig. 2. Rays proceed from each point on the source of light O through the pinhole A to the image I, so that the ray from the top proceeds to the bottom, and *vice versa*. It will also be observed that the size of the image must be in proportion to its distance from the pinhole and the position of the light source. Move the white card further away or the black card nearer the candle, and the image becomes both larger and of necessity fainter.

This simple fact about rays applies to all optical systems. No matter for the moment what the rays are, they travel in straight lines so long as they traverse undisturbed the same medium (air or glass, etc.), and objects are visible by rays from each point which diverge or proceed from that point in all directions (where not stopped by something or the object itself) into any transparent medium.

**Refraction of Rays.**—The rays can readily be reflected into some other direction, as by means of a mirror (image reflection) or by other objects (which we then see by this scattered reflection when not self-luminous). What, however, is of more importance is that they can be bent aside or refracted by the surface and density of any fresh transparent medium through which they then proceed in some other straight line. In such cases it must be remembered that the image is apparently seen in the final direction from which the rays enter the eye. Thus in the looking glass we see an apparent image, and a stick plunged into water appears bent, the lower end appearing higher up than it actually is.

**The Law of Refraction.**—For simplicity in diagrams rays of light are always represented as proceeding in a straight line from the object or light source, but actually this is not the case. Light proceeds in concentric ring waves from any source. As a simple illustration consider a stone thrown into a sheet of calm water. The displacement causes a series of waves spreading out in all directions horizontllay and vertically from the centre.

This is applicable to light, and any point on the diagrammatic line showing its direction may be considered as a wave front and can be treated as a source of light.

These points are essential to the consideration of refraction. Let the lines W $W_1$ in fig. 3 represent a block of glass with air above and below. The line N is *normal* or at right angles to the surface, and light travelling in this line is retarded, but its direction remains unchanged.

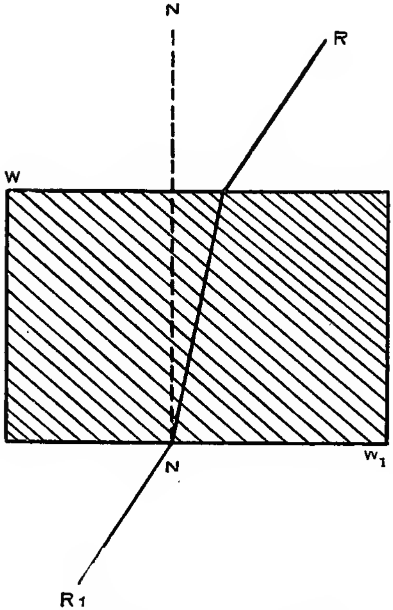


FIG. 3.—Illustrating the Law of Refraction

On the margin of the first wave reaching the denser medium there is a distinct retardation (as, for example, with a man walking in water). This retardation is progressive and consequently alters the whole direction of the light wave. On again reaching air there is a reverse action, the light proceeding at a greater speed and thus attaining a path parallel to its original direction.

Thus we say that light entering a denser medium is refracted towards the normal, but on entering its original medium is refracted back to its original path and emerges parallel to the incident line.

As a simple experiment take a block of glass and place on the one side two pins at an angle to the surface but in a straight line with each other, then looking through the glass at the pins insert two other pins separated by 4 to 6 inches, so that all four are in an apparently continuous line. Remove the glass and join the pinholes with a pencil line, and it will appear as the diagram above.

**Total Reflection : Critical Angle.**—Refraction at a surface takes place up to a spot where the line between two media becomes opaque to the ray. Assume that the air ray 1 grazes the surface of the glass. This surface then becomes the sine. It must be borne in mind that whilst the ratio of reflections remains the same in any one medium whatever the angle of incidence, there is an increasing amount of inclination the greater the angle the incident ray makes from the perpendicular, until arriving at what is known as the 'critical angle,' when the refracted ray cannot emerge from the denser medium to which its refraction is due. This is as a result totally reflected back at the same angle, and is readily demonstrated by holding a glass of water above the eye. The surface looked at from below is of mirror-like brightness and reflections can easily be distinguished in it.

The law of this refraction is of interest and very important. Let us take water and air, and let W $W_1$ (fig. 4) represent the surface of the water, and draw a perpendicular, P *p*, through some point, C, where every ray is to strike the water, and also describe a circle round C. Any ray, P C, striking the surface perpendicularly, goes straight on to *p*. Take some other ray, A C, striking the water at an angle A C P with the perpendicular.[1] Part of it will be reflected at an equal angle, as if the surface were a mirror;

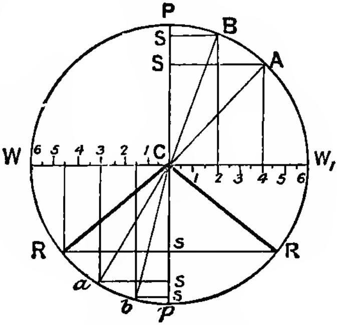


FIG. 4.—Law of Refraction

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[1] Optical angles are measured from the perpendicular, called 'normal,' and not with the surface.

but the part that enters the water is *refracted* at C to *a*, down towards the perpendicular, because the water is denser than the air.[1] That is the first law. In the denser medium the rays are refracted *towards* the perpendicular. Conversely, on entering a rarer medium, they are refracted *from* the perpendicular, so that a ray from *a* to C in the water would be refracted to A. This involves another important fact, that if we trace a ray the other way, its path is exactly the same, or it is *reversible*.

But the measure of the refraction is most important to us. Draw the line A S from where the ray cuts the circle, at right angles to the perpendicular P C; then A S is the geometrical *sine* of the angle A C P. Divide C W and C W$_1$ each into six parts numbered, and let the ray be such that a perpendicular from A meets C W$_1$ at the fourth division: this gives the measure of A S on C W$_1$ as on a scale. Treat the sine *a s* of the refracted ray C *a* in the same way; we shall find A S (in water) measures *three* divisions. A S and *a s* are in the proportion of 4:3. Now take some other inclined ray B C—for convenience we take it here, so that the sine B S of its angle is exactly half A S, or two divisions. Then the refracted ray C *b* will be such that its sine *b s* will also *measure half of a s*. And so with any other rays; the sines of the incident and of the refracted rays are always *in the same proportion* of 4:3.

It is the same with all transparent media, including glass. The proportion differs in each, according to its density, so that in crown glass the ratio is about 3:2. But the ratio is *the same for all rays*, and that enables us to put the ratio into a simpler number compared with air as 1, and call that number the 'refractive index' of the fluid or glass. Thus the index of ordinary optical crown glass is

---

[1] The *optical* density is meant. As a rule heavier fluids or glasses refract more, but not always. Thus oil of turpentine floats on water, but has more optical refractive power.

1·516. And when we know the index, it is easy to calculate the direction in which a ray entering at any angle will go.

The diagram demonstrates, among other things, how fast refraction increases with the angle, and this fact brings us to a curious limit. Let the air ray just graze the surface W $C_1$, then this surface itself is the sine, or 6 divisions. Conversely, the ray R C will just escape from the water in the path C $W_1$. If, then, we take some ray in the water of *greater* angle than R C *p*, what is to happen to that, since it cannot get out of the water? It does not do so, but is *totally reflected* back at the same angle, with a brilliance no mirror can equal; as can be seen by holding high a tumbler of water, and observing the bright reflection from *under* the surface. This reflection at the surface of a rarer medium is the only case of *total* reflection of rays in nature; but the very important point to us is that rays cannot get out of a denser medium, beyond what is called the 'critical angle,' R C R. This angle is plainly less, the denser the medium. In water the critical angle is 97½°, in crown glass 82¼°.

**Action of a Lens.**—The reason that an object is not displaced when looking at it through a sheet of glass, such as a window, will be readily understood from the foregoing description of the law of Refraction, as the rays which on passing through the glass are bent towards the perpendicular, return parallel to their original direction on re-entering the air. If, however, the glass has an inclined surface and unequal thickness, as is the case with a prism, the rays on emerging follow a different path, and are permanently refracted.

Fig. 5 is a diagrammatic drawing of this. The ray R I is refracted towards the normal in the glass from I to E, and on emerging to air is refracted again away from the normal in the line E R, and thus an object R would appear to lie in the direction R O. The ray is always refracted towards the thick end or base of the prism. Not only, however, is a ray of light refracted in this manner, but it is at the same time divided up into its component colours, white light being a mixture of various colours. A three-cornered glass lustre will show this excellently. If a candle flame be observed, the refractive beam of light will be seen to be widened out into a band of colours, which is known as the spectrum.

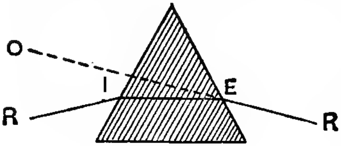

FIG. 5.—Prism

If a lens, with its curved surfaces, be considered as two circular prisms placed inwards, the formation of a focal point will be understood. However, it should be remembered that there are six possible forms of single lens, as shown in fig. 6. The first three are thickest in the centre, and are known as 'positive' lenses, while the last three are thinnest in the centre, and are known as 'negative' lenses.

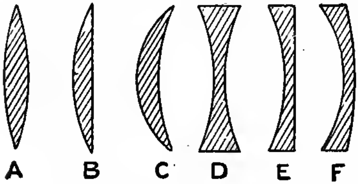

FIG. 6.—Forms of Lenses

To consider the formation of an image from a bi-convex lens: Apply the principle of light passing through a prism, and consider two prisms base inwards, but with curved

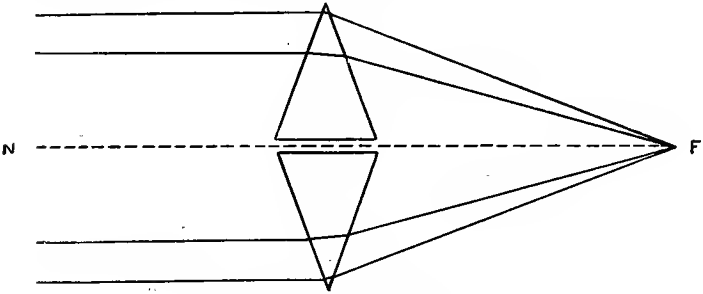

FIG. 7.—Common Magnifying Glass

instead of straight sides. (This is the common form of magnifying glass.) Rays passing along the centre of this lens are undisturbed in their direction, but the refraction increases towards the apex of each of these prisms (fig. 7), and as a result, the rays of light are all brought to a focus at one point. Actually there are several foci, *i.e.* one for red light, one for yellow light, and one for blue light, etc.; but these are very close together. The matter will be referred to further on.

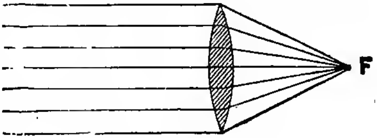

FIG. 8

Fig. 8 shows a parallel front of rays striking on a bi-convex lens. If, however, rays proceed from a source of light at F (fig. 9), they would emerge on the other side of the lens, either as a beam of parallel light or, if the point F lies beyond the focus of the lens, they would on traversing it be brought to a focus either side, and *vice versa*. If these points are interchangeable in this manner, they are known as conjugate foci.

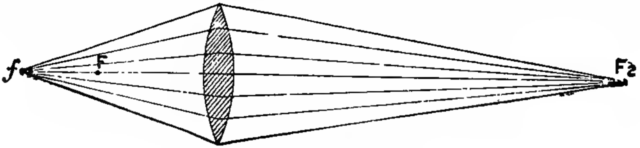

FIG. 9.—Conjugate Foci

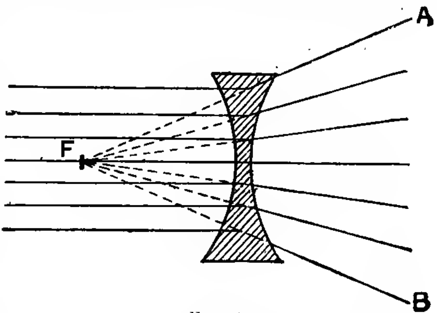

FIG. 10

In the case of concave lenses which are thickest at the edges, the action of the lenses is reversed, and light rays are made to diverge. Such lenses have no actual focus, but what is known as a negative focus. This is measured from parallel rays in the same manner. If the parallel rays falling on the lenses are refracted (fig. 10) as at A B, these rays when produced backwards on the other side of the lens, will meet at the point F. This is known as the 'negative focus.'

**Lenses and Images.**—If a reading glass of 3 inches diameter be taken, a simple experiment will demonstrate the property of a lens in forming images. Take a small source of light in a darkened room and set up a white card screen, as described on page 12. Make three or four pinholes in the black card around the first one, but an inch or less from it, in such a manner that the lens more than covers all the holes made. If the reading glass is about 10 inches focus, as is usual, put the black card 8 inches from the candle with the centre pinhole on a level with the flame, and the white card 3 feet beyond. There will now appear five inverted images of the flame on the white card, showing that each pencil of rays forms its own separate image.

Holding the reading glass close to the apertures, note to what extent the refraction of the lens bends in the four outer images towards the centre one. Move the lens gradually towards the white card, and it will be noticed that they approach more and more until there is a point at which the five images become one. This is the focus of the lens. More holes can be made, or the black card removed, but there will only be one image, which will combine the whole of the ray traversing the black card or the lens should the card be removed.

The function of a convex or image-forming lens is to determine a space where the cone of rays diverging from any point in an object shall recombine in one image. Its position marks the place where the rays are refracted to this end, and also the place where the rays from the opposite point of the object cross and invert the image. Thus, the relative sizes of image and object are measured from the optical centre of the lens according to the ratio of the two conjugate foci. The lens itself, however, does no more than thus converge, and combine into one, potential images formed by the bare rays.

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# CHAPTER II

## PRACTICAL OPTICS OF THE MICROSCOPE

WE come now to certain optical principles belonging to the microscope specially, which should be understood if the best choice is to be made amongst the instruments and accessories offered to our notice.

**The Simple Microscope.**—The action of this instrument, or a simple 'magnifying glass' in any form, will be easily understood after the last chapter. Let the arrow *a d* represent an object O, and L a lens held in front of the eye E (fig. 11). From one point *a* in the object, diverging rays *a b* and *a c* proceed, and are so converged by the lens as to

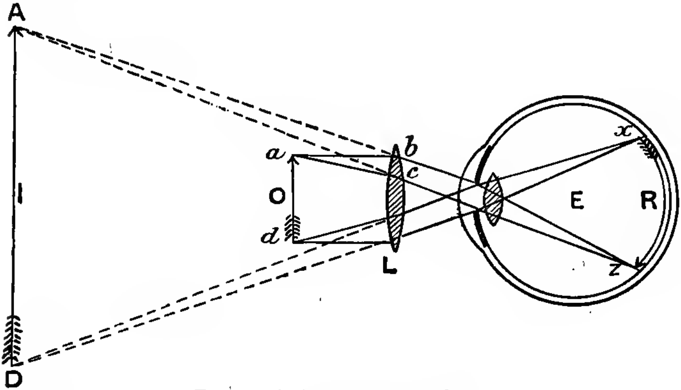

FIG. 11.—Action of Magnifying Glass

enter the pupil of the eye almost parallel, which is required for distinct vision. The rays thus parallelised are brought to a focus at *z* on the retina R, forming there the visual image of *a*. The image of *d* is imaged at *x* in the same way. But the object being 'seen' in the direction of the rays which last enter the eye (see page 13) if we produce these till they meet, they appear to proceed from the points A D, which is the apparent image I of the object, as apparently seen at the distance of 10 inches. It is never to be forgotten that the last stage in every optical combination is the formation of this image on the retina at R, and that the final rays which must enter the pupil in order to effect this, have to be rendered so slightly divergent as to be practically parallel.

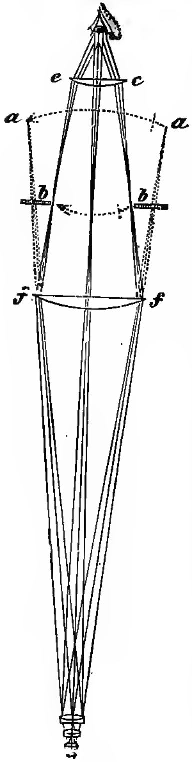

Fig. 12

**The Compound Microscope.**—In this instrument, known in general as *the* microscope, and the general plan of which is shown in fig. 12, a real enlarged image of the object O is formed by one lens (called the object glass or objective) in the air. This enlarged image is looked at through another lens called the ocular or eyepiece, and by it further enlarged in the manner of the simple microscope. For reasons to be seen immediately, each of these lenses is always compound, and may be of various forms; but it is well to notice at once the form of eyepiece shown in the diagram, and called the Huyghenian or 'negative' eyepiece. It is called negative because the image looked at is formed within it, or between its two lenses; and some form of it is more used than any other.

It consists of a larger plano-convex lens *f f*, called the field-lens, a smaller one, *e e*, called the eye-lens, and a diaphragm or stop at *b b*, in the focus of the eye-lens. Three pencils of rays are drawn from the centre and each end of the object o, which if let alone would form an inverted image at *a a*. This aerial image 'in space' behaves in one respect differently from a real object, which sends out wide cones of rays in all directions. The image, on the contrary, is formed by the narrow convergent pencils *o a*, which continue on after meeting or focussing. So if we looked at the image by the lens *e e* alone, only rays from the centre of the image could pass through this lens and reach the eye. But the field-lens bends in the pencils *f a* to *f b*, also shortening the focus a little, forming a rather smaller image at *b b*, and so altering matters that the rays *f a* are now all converged to pass through the eye-lens *e e*. Besides this, the 'aberrations' presently mentioned are nearly corrected by this simple form of eyepiece. And lastly, as the rays begin to be converged later by the edges of the lens *f f* than by the centre, the conjugate focus is rather extended there, and so this eyepiece helps to 'flatten the field.'

It will be obvious that the field-lens *f f* belongs in principle to the objective, taking a share in the focal convergence of the image-forming rays. But it is always reckoned as part of the eye-piece.

**Power of a Microscope.**—This is calculated by multiplying the original magnification of the image *a a* by the further magnifying power of the eyepiece. If an objective of $\frac{1}{4}$-inch focus forms its image at 10 inches, it will at that distance give a magnification 40 times as large as the object o. Then, if the eyepiece magnifies 10 times, the power within that objective and eyepiece will be 400 diameters. But it must be remembered that a tube length of 10 inches is very rarely employed. Thus with a shorter tube length the magnification will be reduced. Most modern objectives are corrected for a tube length of either 6 or 8 inches or 8–10. Thus the magnification is as 6 : 10.

**Spherical Aberration of the Lenses.**—If simple lenses did perfectly the refracting and image-forming work described in Chapter I, a microscope would be simple indeed; unfortunately they do not, failing in three ways.

Firstly, they are ground to spherical surfaces, and these refract too much in proportion as we approach the edges; thus the rays falling on the edge of the lens come to a focus nearer the lens than the central rays. The image formed by a large reading glass on close examination will be found rather blurred from this cause, which is called 'spherical aberration.'

Secondly, it will be noticed that print seen through such a glass appears distorted in the marginal parts. And the image on a card of a flat surface like a lantern slide is not itself flat, but curved hollow towards the lens. These are other effects of spherical aberration.

**Chromatic Aberration.**—Thirdly, in looking through the glass lustre or prism at the candle-flame (Chapter I), the image was not only refracted, but spread into a band showing all the colours of the rainbow—the coloured band called the spectrum. That is because different colours are differently refracted, blue being the most so and red the least. This being so, clearly a simple lens must bring the blue rays to a focus nearer to it than the red rays; and this can also be seen, with care, in any large lens. It is called chromatic aberration.

These faults do not prevent much rough useful work with simple lenses, as in a magnifying glass. But they are fatal to precise work, and most of all to the perfection of images magnified many hundreds of times.

**Correction of Aberrations.**—Such defects can be more or less corrected in several ways.

(*a*) All of them are worst at the edge of a lens. Hence, by stopping all but a portion in the centre, the image is greatly improved in sharpness. But it is at the expense of light and of other functions of large aperture. Hence this plan can only be used to some extent, and as the cheapest mode of improvement.

(*b*) Both aberrations differ greatly in amount, according to the inclination of the surfaces to the rays. The spherical aberration of a plano-convex lens in parallel rays is less than a third when the flat side is turned towards the focus, of what it is the other way. And two inclinations may be so disposed that the aberration of one largely corrects that of another. Thus, by using two or more lenses, amongst which the power is divided, instead of one, and proper combination of curves, much may be done. This method is largely employed, and is entirely depended upon in the Huyghenian eyepiece.

Chiefly of all, while different sorts of glass differ largely in both their refractive power (or optical density) and also in their dispersive power (or degree to which the spectrum is spread out by a prism of given angle), the two qualities do not preserve the same proportion. So it is possible to make, let us say, a plano-convex lens (fig. 13) of two glasses, of which the crown-glass lens, C, has double the curvature of the flint-glass concave lens, F, and so has a large surplus of refractive power, while yet the colour-dispersion of F is enough to counteract all that of C, and so brings all colours nearly to the same focus. Such a lens is called achromatic, or 'corrected for colour.' Fortunately it also happens that the concave curve of the flint lens largely corrects the spherical aberration of C as well; and so we get a more perfect lens every way.

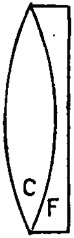

FIG. 13

**Microscope Objectives.**—A lens such as the foregoing is not of sufficient high standard for microscope work. It is possible to correct much but not all of the spherical aberration except in the one case where all the rays proceed from one point in the optic axis, and the conjugate focus is at another point on the axis. This applies principally to cases of telescopes. A better correction can be obtained by employing a single triple combination, a form used with the cheapest microscope low-power objectives, such as the 4-inch, 3-inch, and 2-inch. There are a few 1½-inch, 1-inch, and ⅔-inch objectives made on this principle, but they should be avoided.

A very much better result is obtainable when combinations of two achromatic lenses, having a separation between them, or even more lenses are used. In these different optical glasses are combined, having appropriate curves presenting various angles to the rays, and by this means the beautiful results of modern microscope objectives are made possible.

Although similar results are obtained by different makers, this does not mean that their combinations are identical. Each maker has his own method of correcting his lenses, and applying the use of various glasses. Thus for a ½-inch lens three opticians may use entirely different glasses, different curves, and different separations. These objectives are not the result of trial and error, but of definite mathematical computations based on theory. It will be understood that lenses may vary greatly in their construction, and consequently in their first cost, owing to complications. It must not be considered that the higher cost is not worth while. Often, as appreciation of the optical results obtainable with the microscope increases, the value of the best objectives obtainable will be realised.

**Effect of Cover Glass.**—If the correction still leaves the focus of the marginal rays shorter than that of the central rays, it is called 'under-corrected.' If, on the other hand, the correction is so great that the central rays have a shorter focal point than the marginal rays, it is called 'over-corrected.' If one makes an objective aplanatic and achromatic for the observation of an object without a cover glass, it will be realised from fig. 14 that the interposition of a cover glass throws all the corrections out of balance. Let L in fig. 14 be an object in balsam B, under the cover glass C, and O P P, also O Q Q two pairs of rays proceeding at large and small angles from O. At P P and Q Q they are refracted away from the normal, as shown. Producing back these rays, as they enter the lens, it will be seen that it is really as if one pair proceeded from A, and the more marginal rays, as if they proceeded from B, nearer the lens. Such foci for marginal and central rays represent under-correction, and hence the objective, to focus truly under these circumstances, must itself be under-corrected for rays proceeding from O in air. Also the thicker the cover and the mounting medium above the object, the more this effect will be exaggerated.

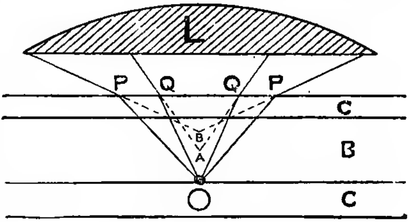

FIG. 14.—Effect of Cover Glass

This brings up a most important point known as Tube Length Correction, which plays a vital part in the formation of accurate microscope images, and to which reference will be made further on. In any objective with two or more components in the optical system, under-correction can be produced by approaching the front and back combinations to each other conversely. Over-correction is produced by separating them. This is the reason for the introduction of the Correction Collar on microscope objectives. This is now rarely met with, as the theory of tube length correction of cover glasses is better understood, and it only achieves the same result. But it must always be remembered that the better the correction of the objective the more sensitive it is to cover glass thickness, an error of less than 10 mm. in the tube length being sufficient to ruin the image.

**Angle and Aperture.**—In the past, before the function of numerical aperture was described by Professor Abbe, and before immersion objectives came into use, lenses were described by their angular aperture. This definition has now been completely discontinued, and lenses are always described by their numerical aperture, following on the formula laid down by Professor Abbe. This is arrived at by taking the angular aperture of the lens and measuring the actual diameters of the pencils of rays transmitted, which depends more upon the back of the lens than the front.

For comparative purposes, a geometric measure was selected, and this was the radius or half diameter, the relative proportion of which would be the same, and which geometrically is the sine of the semi-angle of the outermost rays transmitted from the objective. It was shown that if this sine or half the outside angle were multiplied by the refractive index of the medium used, a constant would be arrived at, giving the comparative aperture of any lens whatever the medium. This is known as the numerical aperture. The formula is:

$$\sin U \times N = N.A.$$

where U is the semi-angle and N the refractive index of the medium.

**Immersion Objectives.**—These objectives are in constant use nowadays, and form part of every microscopist's outfit. But it is seldom realised that they are quite a recent innovation, the credit of which is due to Mr. Wenham. Immersion oil supplied by microscope objective makers is a mixture of cedar wood oil and other substances, producing a fluid having a refractive index as near as possible to that of the glass, viz. 1·516, so that the diffusion of the ray passing from the condenser through the slip object and cover glass continues unaltered in the objectives. This will be clearly understood from fig. 15. An interesting point is that though theoretically cover glass thickness does not count

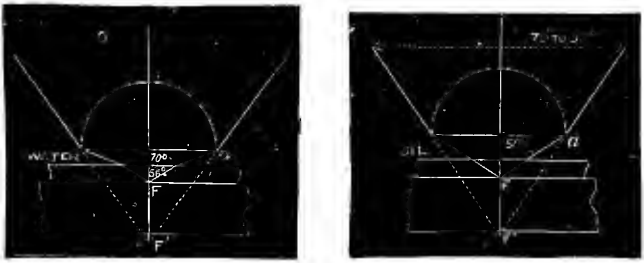

FIG. 15

when an objective corrected for oil immersion is employed, yet equal care must be given to tube length adjustment.

**Aperture and Resolution.**—An idea has prevailed, and still prevails, that to 'see' advantageously with a microscope lens, high magnification must be employed. This is an entirely mistaken notion. The power to 'see' fine detail depends entirely upon the numerical aperture of the objective, and the eyepiece only magnifies the image formed by the objective.

The term 'resolution,' as applied to the optical equipment of a microscope, is self-explanatory. It signifies the power of a lens system to split up a series of fine lines, so that each can be independently viewed. This power is entirely dependent upon numerical aperture or the angle of the lens. A simple experiment will easily demonstrate this. From a distance observe a venetian blind and endeavour with the eyes alone to count the laths in the blind. This will be found impossible, as they tend to blur together. Now, advance to within 4 or 6 feet of the blind, and the quantity of laths composing it can easily be counted. This is owing to the fact that the angle subtended by the laths to the eye, is greater the nearer the observer approaches the object examined. If this example is applied to a microscope lens, the meaning of the expression 'power to resolve lines according to aperture' will be immediately comprehended, and however great the magnification given by the eyepiece there will be no improvement of the resolution.

The result of the work of Professor Abbe has conclusively demonstrated the uselessness of high-power objectives manufactured until recent years. Modern apochromatic oil immersion objectives of $\frac{1}{8}$-inch to $\frac{1}{12}$-inch focus, having numerical apertures of 1·3 to 1·4, give all the resolution obtainable, and they have the advantage that they will bear the deepest eyepiecing, if properly computed, to an extent hitherto unknown. Owing to the variation in makers' descriptions of their objectives, specifications in angular and numerical aperture, the need of some simple formula for expressing relative values of lenses is felt. As a result Mr. Nelson some years ago suggested a standard which shows the real value of any objective in figures, which is known as the optical index of the objective.

This factor is obtained by multiplying the numerical aperture by 1,000, and dividing the result by the initial magnifying power of the objective. For instance, a $\frac{1}{2}$-inch objective with an initial magnifying power of 20 diameters 0·65 n.a. will have as its optical index:

$$\frac{0{\cdot}65 \times 1{,}000}{20} = 32{\cdot}5$$

Reverting to the uselessness of high magnification, take as a comparison one of the old objectives, say $\frac{1}{50}$th of an inch, n.a. 1·1:

$$\frac{1{\cdot}1 \times 1{,}000}{500} = 2{\cdot}2$$

The comparison between this and the modern $\frac{1}{2}$-inch objective is striking. The old $\frac{1}{50}$th of an inch lens possessed a vast amount of empty magnifying power. Theoretically, an objective should have 0·26 n.a. for each 100 diameters of combined magnification, but in practice less than this is found sufficient.

Confusion often arises between the equivalent focus marked on the mount of objectives and their actual working distance. This equivalent focus does not represent their working distance, but indicates solely the magnifying power. Thus the unit lens may be termed a 1 inch, and has a magnifying power of 10 at a distance of 10 inches from its back lens. A $\frac{1}{2}$ inch on this scale will give 20 diameters, a $\frac{1}{4}$ inch 40 diameters, and so on.

For apochromatic objectives and other fine quality lenses decimal measurements are usually employed, and the unit is a lens of 24 mm. Thus a 24-mm. objective gives approximately 10·4 diameters at a distance of 250 mm. from the back lens, and will be found nearly approaching in magnification an objective of 1 inch of the achromatic series.

Frequently, owing to the German method of not engraving the focal length or apertures on their objectives, one comes into possession of an objective of unknown focal length. To determine this, however, project an image of known size, say the lines of a stage micrometer, on to a screen 10 inches distance from the back lens, and ascertain the size of the projected image by means of a ruler. Divide this size into 10, and the so-called focal length will be ascertained.

The same applies to ascertaining the initial magnifying power of an objective described in mm. Thus: divide the focal length into 250, and a 4-mm. objective should give approximately 62 diameters. Unfortunately a habit has prevailed, and still obtains with some makers, of marking their objectives with a lower focal length than is justified by the actual magnification of the lens. For instance, an objective marked 1 inch, in some series often gives an initial magnification of 15 diameters, being really a $\frac{2}{3}$ inch. There are in circulation $\frac{1}{4}$ inch, which are really $\frac{1}{6}$-inch objectives, but the worst offenders are those who put into circulation objectives engraved $\frac{1}{6}$ inch, which in reality are $\frac{1}{8}$ inch.

Care should always be taken in purchasing an objective, that it bears engraved its focal length and numerical aperture. These are a guarantee which can be at any time checked by the methods described.

To those accustomed to use the microscope it is well known that objectives are engraved in this manner so as to convey the idea that they possess a numerical aperture well proportioned to the magnifying power, or in other words a high optical index. For instance, an objective engraved as a $\frac{1}{4}$ inch with n.a. of 0·68 has on the formula shown an optical index of 17, but if actually this $\frac{1}{4}$-inch objective has a magnification of a $\frac{1}{6}$ inch, the optical index is only about 11·3, which is a very different matter.

As we have hinted earlier, certain manufacturers are continual offenders in this respect, but this reproach does not amongst others apply to W. Watson's objectives. All those that I have examined are extremely near to their focal length as engraved on the mount, and they usually understate their numerical apertures. German makers have a practice of simply designating their objectives by arbitrary numbers without marking the focal lengths or numerical apertures. There is nothing to recommend this practice, which is confusing and misleading.

In selecting objectives the first process should be to obtain the makers' catalogues, compare the stated magnifications and numerical apertures, and so far as may be possible, limit the selection of one's objectives and spend the money available by buying the best possible. It is very important to understand the real meaning of aperture, in regard to microscope objectives. This is largely bound up with the theory of microscopic vision, as enunciated by Professor Abbe, and known as the 'Diffraction Theory.'

Before Professor Abbe investigated this question, it was always assumed that the formation of microscopic images was in accordance with ordinary dioptric principles. In other words, that the image in the microscope was formed similarly to that in the telescope. Aperture, of course, is necessary in the telescope, but that is simply the measure of the diameter of the object glass. Such measurements cannot be applied to microscope objectives, as the front lens of a $\frac{1}{12}$-inch oil immersion objective may measure a small fraction of an inch in diameter, while that of a 1-inch objective may be as large as $\frac{1}{4}$ or $\frac{1}{3}$ of an inch in diameter. Yet the $\frac{1}{12}$-inch objective possesses far more aperture than the 1 inch. So long as microscope objectives were made to work without any immersion medium, the measure of the angle of the cone of rays that they could embrace from an object gave an accurate comparison of various lenses, and this measurement was termed the angular aperture. The introduction of water, oil, and other immersion lenses soon demonstrated the futility of such measurements, as a dry objective possessing a theoretical angular aperture of 180° was equivalent to one with an angle in water of 96° and only 82° when immersed in oil. In fact, it was soon realised that lenses in different media might possess the same angle whilst the real apertures were vastly different.

Professor Abbe demonstrated that the real definition of aperture is the comparison of the diameter of the emergent pencil of rays from the objective and its focal length.

As mentioned previously, microscope objectives are not simple lenses, but are composed of a complex optical system, and such emergent pencils therefore are a measure of the effective diameter of the back lens. The principle adopted by Abbe was that there existed a general relation between the pencil of light taken in by the front of the objective, and that emerging from its back lens. As mentioned in the paragraph on numerical aperture, the ratio of the semi-diameter of this emergent pencil to the focal length of the objective can be expressed by the sine of the semi-angle of aperture multiplied by the refractive index of the medium in which the objective works, *i.e.* air, water, or oil. This is stated as N sin U = N.A., U being the semi-angle of aperture; N the refractive index of the medium.

It was a question why increased aperture was necessary to define minute structure which led Abbe to formulate the theory known as the 'Diffraction Theory.' He was able to show that the formation of a microscopic image did not take place in accordance with ordinary dioptric laws, but was dependent upon diffraction effects. The rays of light falling on an object are broken up into other groups of rays giving a direct pencil and several lateral or diffracted pencils of light (see fig. 29). With a coarse object the diffracted rays are very little separated from the direct or incident rays, and usually escape notice except with objectives of very long focus.

Both incident and diffracted rays are consequently admitted into the objective together, and the image is exactly similar to the object. In excessively minute structure, however, such as diatom markings to be found on Pleurosigma angulatum, Amphipleura pellucida, etc., there is a form of double separation—firstly, the direct or incident rays which hold the main outline and coarser structure of the object, and, secondly, the diffracted or bent-off rays which correspond with the minute detail.

It follows, therefore, that a lens of small numerical aperture will fail to embrace these diffracted rays, and in such a case the detail becomes invisible, and only the outline of the coarser markings are shown. The smaller the detail, the further from the direct rays will the diffracted rays be bent, and lenses of greater or higher numerical aperture must be employed to grasp these diffraction spectra, if result is to be obtained.

The diffraction spectra so far can easily be seen in examining the well-known diatom *Pleurosigma Angulatum* with a $\frac{1}{6}$-inch objective having a numerical aperture of 0·70 or more. Having placed the specimen in the centre of the field, remove the eyepiece and examine the back lens of the objective. On closing down the diaphragm to approximately one-half of the diameter of the back lens the central beam will be seen surrounded by six bluish-green coloured circles equally placed round the margin of the back lens. These circles represent the diffracted beams, and the formation of the microscopic image can then be explained by the fact that the direct and diffracted rays which are seen at the back of the objective produce interference phenomena, which in turn reveal the detail seen by the eyepiece.

Another point to which sufficient attention is not yet paid in the formation of microscopic images, is the part played by the condenser, to which reference will be made further on.

**Apochromatic Objectives.**—The most recent advance in the optics of the microscope is the apochromatic objective. This is a series of lenses in which the computation is such that the secondary spectrum is completely eliminated, generally by the employment of fluorspar. To these further reference will be made in Chapter IV. It should, however, be pointed out that with one exception these objectives are computed in such a manner that they cannot be employed except with specially designed eyepieces, known as compensating eyepieces.

In order to facilitate the correction of the objective, so as to eliminate the secondary spectrum, the lenses are what is termed 'under-corrected,' *i.e.* the colour is not fully eliminated. They are then used with eyepieces which are over-corrected, and which balance up exactly the under-correction of the objective. For this purpose, if a firm making apochromatic objectives lists the special compensating eyepieces to be used with them, although theoretically, they may be worked with other makers' apochromatic objectives, unfortunately such eyepieces only work to the best advantage upon the makers' own objectives, for which they are computed, and consequently if several apochromatic objectives of various makers are in the microscopist's battery, he should have a series of compensating eyepieces by each maker whose apochromatics he possesses.

To overcome this difficulty W. Watson & Sons designed and produced their Holoscopic series of eyepieces, with magnifications × 5, × 7, × 10, × 14, and × 20. These are provided with a sliding tube adjustment, so that the field and eye lens can be separated, and consequently the desired amount of under-correction obtained. With these eyepieces it is possible to use all forms of apochromatic objectives to their best advantage.

The following is a table of some numerical apertures, showing the respective angular pencils which they express in air, water, and cedar-oil or glass. Of the higher powers it is most convenient to take round numbers in n.a., and their angles can only be stated in fluid media; of lower powers it will be most serviceable to give the nearest n.a. equivalents of the more usual angles used in 'dry' lenses.

| N.A. | Air angle | Water angle | Oil angle |
|---|---|---|---|
| 1·40 | — | — | 134° 10′ |
| 1·30 | — | 155° 38′ | 117° 35′ |
| 1·25 | — | 140° 3′ | 110° 59′ |
| 1·20 | — | 128° 55′ | 104° 15′ |
| 1· 0 | 180° | 97° 31′ | 82° 17′ |
| 0·95 | 143° 36′ | 91° 10′ | 77° 22′ |
| 0·87 | 120° 55′ | 81° 42′ | 69° 49′ |
| 0·85 | 116° 25′ | 79° 37′ | 68° |
| 0·82 | 110° | | |
| 0·77 | 100° | | |
| 0·71 | 90° | | |
| 0·64 | 80° | | |
| 0·50 | 60° | | |
| 0·42 | 50° | | |
| 0·35 | 40° | | |
| 0·30 | 35° | | |
| 0·26 | 30° | | |
| 0·22 | 25° | | |
| 0·18 | 20° | | |

# CHAPTER III

## THE SIMPLE MICROSCOPE AND ITS USES

Some definition of the simple microscope as compared with the compound microscope is necessary, and in this chapter we propose confining ourselves to the simple microscope, dealing principally with its forms and practical uses.

A single lens or compound series of lenses is said to be a simple microscope, when the image formed by these lenses enters direct into the eye, and is not first viewed or magnified by another lens or eyepiece.

**Single and Compound Lenses.**—Single lenses are constantly used by engravers, watchmakers, and others, but the well-known pattern, known as the watchmakers' eyeglass, which they employ, gives only a low magnification, and its action will be understood from fig. 7. In this case the lens is mounted in an ebonite or horn cell, of cup shape, which is held in position in front of the eye, by the eyebrow.

The more useful magnifier is the botanical pattern, illustrated by fig. 16, which can be supplied with one, two, or three lenses at choice, and is mounted both in ebonite, horn, and tortoiseshell. In this the central hole which is shown in the diagram can be used as a diaphragm, when the lenses are folded back into the mount, and the centre of best definition of these lenses is thus obtained. These lenses at the present time vary in price

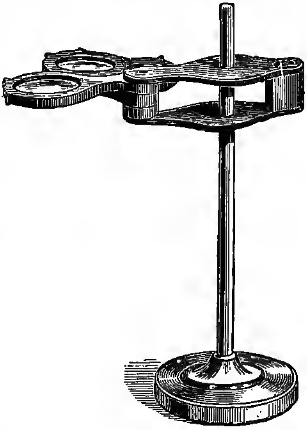

Fig. 16

from 3*s.* 6*d.* upwards, according to the quantity of lenses, quality, and mounting. There are several objections to these—(*a*) The hole in the centre allows dust to accumulate on the lens. (*b*) The mounting is easily broken. (*c*) The lenses are totally uncorrected, giving consequently a curved field and coloured image, while for high-power work the working distance is so close as to render them useless.

For rough examination, however, or for a beginner, they will be found useful.

Here we may mention that the diameter of the lens is dependent upon its magnification, and the greater the magnification the smaller the diameter of the lens, owing to the necessary curve which has to be ground upon it.

The modification of the simple lens made for rather higher powers is known as the Coddington form. This is a thick spherical lens, which has a deep groove ground out around its diameter at the centre. This groove is filled with a black material so that light only passes through the small aperture left at the centre of the spectrum, and thus the effect of a diaphragm is obtained. Fig. 17 shows a mount having a Coddington lens at one end, three plain lenses at the other, having two stops which can be used in between them, but their vogue is over. The best lens of all, and the one at present in most constant use, is the achromatic form introduced by Steinheil, and to-day known everywhere as an 'aplanatic magnifier.' This consists essentially of a triple combination in the form of fig. 18, which is computed to give a wide flat field, with the maximum of aplanatic aperture and working distance. It is offered mounted either in a round metal cell, with a small milling at the top for

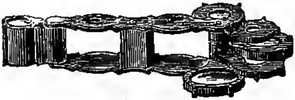

FIG. 17

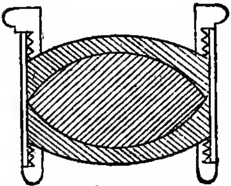

FIG. 18

insertion in a dissecting microscope. Alternatively, in a metal handle, similar to that shown in fig. 19. These lenses can be obtained from all opticians, and their prices are approximately 17*s.* 6*d.* for the magnifier, in dissecting mount, and 21*s.* for the magnifier in pocket mount. The magnifications vary from ×6 to ×20.

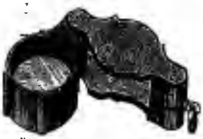

FIG. 19

The most satisfactory plan is to have these lenses mounted in such a manner that they can be used for both purposes. This is achieved by a special mount known as the combination pattern. It is in the form of a mount, having a spring ring of the size to take the dissecting mount magnifiers. For those possessing magnifiers for use in a dissecting

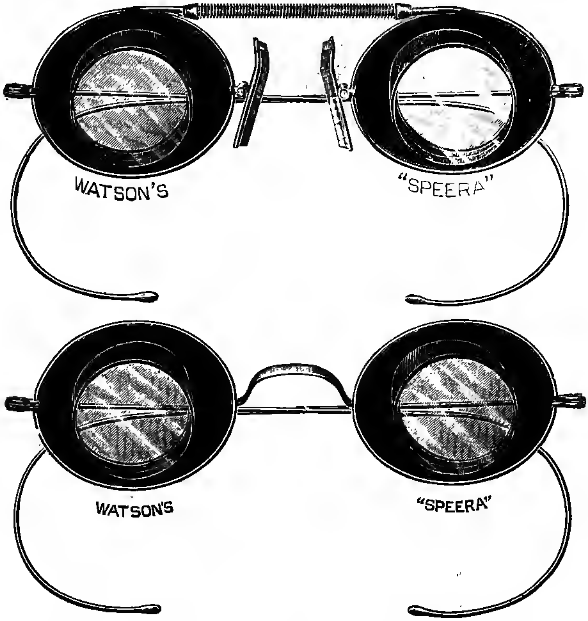

FIG. 20

microscope, it is only necessary to purchase this combination mount, to have the equivalent of a complete battery of magnifiers when on a field expedition.

These aplanatic magnifiers cost little more than the Coddington pattern, but the performance is so superior that relatively they are cheaper. Browning's platyscopic lens on these lines is deserving of special mention.

A new form of stereoscopic magnifier has just been introduced by W. Watson & Sons, and is sold by them as the 'Speera' magnifiers (fig. 20). These consist of two aplanatic lenses, having a combined prism to give the necessary converging power. They are mounted on spectacle frames either of the plain w-bridge type, or the spring bar. As an alternative also, they can be mounted in special funnels to fit on the spectacles of the user. It is claimed for these magnifiers that the aplanatic lenses give freedom from colour, while the use of two eyes yields stereoscopic vision and allows a greater portion of detail.

**Dissecting Microscopes.**—If a single lens is mounted in such a manner that it can be focussed by rack and pinion over a convenient stage, on which the slide or object can be placed and with a mirror underneath which moves freely from all ways so as to throw light on the object through an aperture in the stage, the lens is then converted into a real instrument, with which serious work can be done.

Such a stand is found in fig. 21, and is known as a dissecting microscope. Well made, with a firm horseshoe base, upright pillar, in which moves the rackwork triangular bar supporting the arm which carries the lens, with a firm stage to which are attached rests for the stage when dissecting, and mirror, this instrument should be used by every microscopist. The side rests for the hands depend at an angle of about 30° from the stage, and being detachable are not shown in the figure.

These microscopes are obtainable in two sizes, from

Leitz, C. Baker, and Watson & Sons. They will take all forms of standard-sized dissecting magnifiers, and their prices are £2 15*s*. and £6 for the small and large sizes respectively.

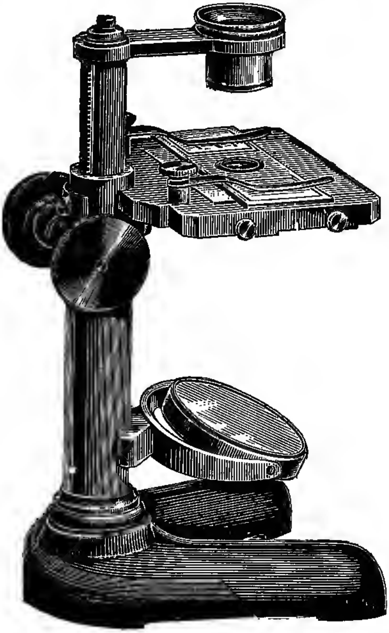

Fig. 21.—Dissecting Microscope

Another invaluable accessory is the Porro prism erector, which fits into the ring provided for carrying the magnifiers, and converts the instrument into a compound dissecting microscope, giving an erect image. Magnifications from ×20 to ×100 can be obtained, combined with a long working distance, sufficient to allow of dissection with needles or fine knives. Objectives 2 inches to ½-inch focal length can be used, and with this addition the most serious dissections can be undertaken. The price of this accessory is £3 10*s*.

**Use of the Simple Microscope.**—No microscopist who has the root of the matter in him ever goes out without a pocket lens of some kind; and a person ignorant of its use has no idea of what can be seen with it, or of the additional interest it is capable of imparting to the world of nature around him. Did I wish to awaken in a young person of either sex such an interest in that world as might probably make the microscope a means of enjoyment and improvement for life, my first present would not be a compound instrument, which might very probably be 'played with' a few times and then tired of, but rather a good pocket lens, with a little practical teaching of what could be seen with it during any walk in the fields.

Take it, for instance, as a friend who will reveal countless hidden beauties in flowers. First, use it to examine the very smallest, which to the naked eye exhibit practically nothing: such as the tiny white florets of fool's parsley, wild carrots, and others of similar magnitude, not forgetting in the season the exquisite 'flowering' on 'tail' and other grasses.

It is wonderful at first to see how, under a simple magnifier, exquisite lilies start into being. Examine also the parts of larger 'composite' flowers; see how each so-called 'petal' of a daisy or feverfew is in truth a perfect little floret by itself; how a head of clover is 'made'; a thistle flower; and others of that sort.

Then let larger objects have attention; it will be marvellous to see the added beauties which appear in such familiar flowers as the forget-me-not, pimpernel, agrimony, heaths of all sorts, valerian, sainfoin; and even in flowers so large as lily of the valley, the hairy yellow St. John's wort, or the various forms of orchis.

This is no attempt at a list; let the owner of a pocket lens, during one walk, simply make a point of looking at every smallish flower or grass in flower that he can find. The small flower thus magnified is not merely like a larger one; there will be seen an exquisite beauty and wavy texture, in most, that only the finest lilies resemble on a larger scale; and many a common flower—the hedge stachys is another good example—puts on a magnificence of decoration absolutely enchanting.

Then the reproductive organs of larger blossoms will repay examination. Though but a portion can be seen of the beauties of anthers, stigmas, and pollen grains, enough will be glimpsed to awaken interest and curiosity to know more; and it is such living interest, and desire to look into things, which makes the real microscopist.

Yet another world of interest will begin to open as various mosses and lichens are examined; and something of the lovely miniature forest contained in a spot of 'mould' may be understood, even from a pocket lens.

A second class of objects likely to interest an intelligent person will be found in the insect world. Tiny insects no larger than a pin's head, only specks to the unaided eye, grow into wonder and beauty under the lens. Little iridescent beetles and flies will exhibit a magnificent wealth of colour and ornament never imagined. With the higher powers, the curious variety of insect organs can be made out, and will add tremendous interest to any popular book on the subject.

The heads of insects alone, with their many organs, so totally different from those of all other animals, will furnish interesting study for months. They are easily examined by preparing a few cones of gummed paper wrapped round and round, and trimmed evenly at the ends, of various sizes, as fig. 22. The insect being inserted head first in one of suitable size, so that it can get its head outside, and no more, a little tuft of cotton-wool is put in behind and held by the end of a finger, when the head can be examined comfortably. For weevils and beetles the cone is first pinched oval, or nearly flat, so as to fit roughly the section of the body. From personal experience I can affirm that even experienced microscopists, previously unfamiliar with it, have found absorbing interest in this method of examination, which is within the reach of any boy with a shilling magnifier. Then the insects, killed as usual in a chloroform or laurel bottle, may be cut up and their organs examined in detail.

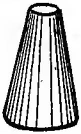

FIG. 22

Something can be seen with a pocket lens even of microscopic pond-life, but this mostly requires more or less training both of eye and search (see Chapter VIII). Such is not, however, the case with sea-life, and the lens will add great interest to a seaside holiday. Take a few glass tubes, with corks, and in these place for examination, with clean sea-water, bits of weed or rock that seem to have anything on them different from the mere weed, especially anything in the shape of slender white or creamy branches, no thicker than small or large pins. After a gale, even at places where there are no rocks, such can be found in great variety on weed washed up by the waves; and in the tube, under the lens, they will be seen to be lovely Hydrozoa. Abundance of small crustaceans, and curious sea-worms, etc., will also be found, which cannot be detailed here.

Something might be added about examining even bits of stones, fresh broken; a bit of granite, for instance, will show plainly how complex is its structure. Seeds, spores, insect eggs, etc., may also receive attention. But more is unnecessary. It is only desired here to indicate how to use a simple microscope. Any one who has so used it, more or less constantly, through one summer season, will have a far better idea of the world of minute nature, a truer conception of its wonders, and most of all, a more vivid idea of how much there is to repay further examination and study, than many a one who has had presented to him a costly instrument and box of objects, and who having looked over those objects, has perhaps laid the whole aside. He may probably have learnt to value the microscope for what it really is, and be fairly on the way to become a microscopist.

The use of the simple microscope in dissecting belongs chiefly to a later stage.

# CHAPTER IV

## THE COMPOUND MICROSCOPE AND ACCESSORIES

GREAT changes have taken place in the microscope since the late Rev. J. G. Wood wrote that a first-rate one cost 'forty or fifty pounds and upwards,' and recommended, as all that could be desired, a three-guinea instrument 'not half the price of a single object-glass belonging to the larger microscopes.'

From about the year 1880 especially, development began to settle into one definite line; the result of which, with the improvement in lenses already sketched in Chapter II, has given great economy, combined with so much greater excellence, that better results can now be accomplished with £15 or £30 than with the £50 above quoted in former years. Even the very cheapest instruments have shared in this progress; but the greatest gains have been in those of a medium quality. Nearly all the old patterns have disappeared, replaced by new ones, which at first sight seem so various, that a novice may not perceive the one general line of development, unless it is pointed out to him.

**The Modern Microscope.**—There is no comparison between the stands manufactured thirty years ago and those made to-day. The modern stand, that any maker of repute produces, has a steadier fine adjustment for precise focussing, and will be found entirely different in its conception and manufacture. This has been necessitated owing to the alterations and improvements in the ordinary achromatic lenses in use to-day, while the advent of the apochromats and the proper appreciation of numerical aperture have widened the whole of the microscope horizon.

Comparing textbooks of 40 to 50 years ago, a vast array of accessories such as spot lenses, Lieberkuhns, reflectors, etc., have vanished. The modern apparatus taking their place has been improved to such an extent that these adventitious aids are unnecessary. To take one instance—the condenser. Fifty years ago wheel diaphragms were in general use, and with the lenses then made the difference was not remarked. With the improvement of objectives came the necessity for an increased illumination which had to be axial. This in turn caused the intervention of the Abbe illuminator, which is provided with an iris diaphragm, so that a graduated diminution of light can be obtained.

Even to-day sufficient attention is not paid to the importance of the condenser, and there is a marked difference noticed when a condenser is used intelligently in the manipulation of the microscope. Further on, particular attention is given to this.

**Choice of a Microscope.**—In choosing a microscope it is absolutely essential to confine one's selection to instruments by makers of standing. The best microscopes are made by four English firms, two American, two German, and one Austrian. There are other small manufacturers; but a firm with a reputation has to supply goods which it is to be hoped will maintain that reputation. The same value, which is the criterion of real cheapness, cannot be obtained elsewhere.

All these makers supply microscopes of various designs and varying movements. The first step is therefore to consider the relative importance of the movements offered. As a practical worker I think that if a concensus of opinion were taken they would be placed in the following order:

(1) Rackwork coarse adjustment.
(2) Substage.
(3) A reliable fine adjustment.
(4) Centring and focussing adjustment to the substage.
(5) Mechanical stage.
(6) Divisions to movements of stage.

(7) Rackwork to draw tube.

(8) Fine adjustment to substage.

Bearing in mind the above arrangement, the microscopes can be ranged in three categories, the first comprising instruments designed for the most critical form of research work. Such would possess all the adjustments given in our list. The second category would appeal to bacteriologists, and would be used by advanced students. Such instruments would be provided with a coarse adjustment by rack and pinion, a substage provided with rackwork focussing and screw centring; a fine adjustment, and a mechanical stage with divisions to its movements, also a sliding bar. Instruments of the third class would possess a coarse adjustment by rack and pinion, a fine adjustment, and a substage, preferably with rackwork adjustment and centring screws. The stage would be plain.

Leaving for the moment the construction of various stands, and their specific details, a few points must be remembered when choosing any stand.

On examining the bearings, there should not be any surplus grease. There may be traces of a slight amount of oil or lubricant, to prevent the metal bearings, when first made, from seizing up, but should there be any excess there is always a risk that the bearing relies largely upon its surplus grease, and this should arouse suspicion. The stage should be firm, and as large as possible. Bear in mind that for medical purposes particularly, a student will want to use Petri dishes on this stage, and it should be at least 4 inches square. The modern students' microscope is generally provided with a stage 4¼ inches square. In addition, the stage should be covered with vulcanite, as reagents can be used without fear of spoiling its surface. This vulcanite to be fitted successfully should be moulded upon the stage, and cover both the upper and lower surfaces of plain stages.

The first and most important points, however, are the coarse and fine adjustments. These should work with perfect smoothness and delicacy, and there should be no shake. To test for this, grasp the microscope limb firmly with the left hand and the body tube with the right one. With a rocking movement, move the body from side to side. If any knock or shake is felt, reject the instrument. It is not fair to test for this with the body tube racked up to its fullest extent, but there should be no shake when the coarse adjustment is engaged half-way over its total bearing.

To test the fine adjustment, incline the microscope to a horizontal position. Focus a $\frac{1}{6}$-inch objective on an object, and then use the fine adjustment. This should be responsive with the $\frac{1}{6}$-inch objective to $\frac{1}{10}$th of a rotation of the milled head. Having gone past the point of focus, refocus with the coarse adjustment. Again try the fine adjustment, Roking in this manner from one extreme limit to the other.

In the coarse adjustment, if there is any stiffness of motion, this is often capable of adjustment, as different microscope users prefer different types of movements, but for usual work a very light coarse adjustment, capable of being actuated by the finger and thumb, is preferable, and when seen like this, there must be no shake and no tendency whan the nosepiece and objectives are in position for the body tube to 'run down.' All coarse adjustments should be capable of focussing a $\frac{1}{6}$-inch objective, without the assistance of the fine adjustment, and this latter should never be used with powers less than a $\frac{1}{6}$ inch.

Another point in connection with the coarse adjustment is that it must not only be capable of focussing high powers accurately, but it must have sufficient range to focus low-power objectives. A microscope which gives its owner a latitude of focussing objectives from 1 inch focal length to $\frac{1}{12}$ inch, is not universal in its application. There must be latitude for focussing a 2-inch objective in position on the nosepiece, and for still lower power objectives, such as the

3-inch, 4-inch, and photo-micrographic lenses, the draw tube should have fitted at the opposite end to the eyepiece a diaphragm with the R.M.S. standard objective thread cut on it.

Next examine the diameter of the body tube. This should not be less than 35 mm. for all-round work. The purchase of a microscope is not an annual affair, and such instruments as are bought should be bought bearing in mind their capabilities of adaptation for any form of work the owner may wish to undertake.

In this connection, economy on the microscope stand is a false economy. Always purchase the best stand you can afford, even if it is necessary to buy fewer objectives in the commencement. These can be added later, but the microscope stand is a lasting purchase, and unless its possessor has a feeling of comfort in using it, and pride of ownership, it is useless.

In this connection always examine the microscope, to see that a compound substage, *i.e.* one with rackwork focussing and screw centring movements can be added later, as these will become necessary for serious work. If possible, buy a microscope provided with this fitting.

**Parts of a Microscope.**—In fig. 23 will be found an illustration of a microscope which is lettered, and this refers to the next section. The foot K is the base upon which the whole instrument stands, and the feet can be divided into two types—the tripod, of which there are many variations, and the horseshoe pattern. These are also known as the English, and Continental. Undoubtedly the best form is the tripod, for scientific weight distribution and rigidity, and this has been continually referred to by microscopists. Unfortunately, there has been a prejudice against it, and beginners are often advised by friends of but slight knowledge to purchase a Continental pattern foot. The only objection to the tripod foot is that a somewhat larger case is required, but for constant critical work, visually or photomicrographically, it has no rival. It is steady in whatever position it is placed, and remarkably free from vibration.

The horseshoe, or Continental pattern foot, is more portable, owing to the fact that it is compact, but a large amount of steadiness is lost, except in the upright position.

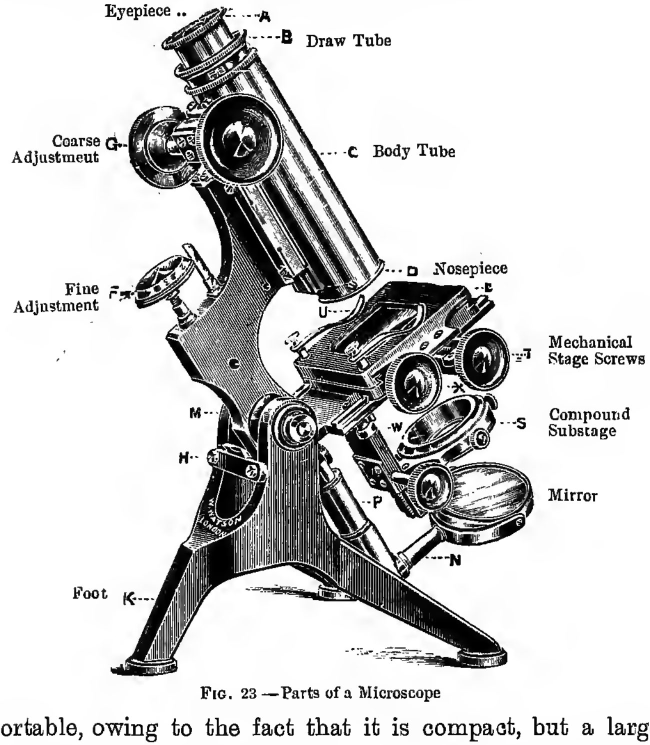

FIG. 23.—Parts of a Microscope

The fact that in the majority of laboratories a microscope is used in a vertical position, accounts for the slight amount of attention paid by most manufacturers to the rigidity of this pattern. As we have stated, it is a favourite in many laboratories, owing to the incomprehensible prejudices which have existed in the past in favour of the Continental stand.

The horseshoe pattern feet at present offered are considerably steadier than those made ten years ago, and if an instrument thus formed is decided upon, it should not be purchased unless the microscope can be inclined so that the body is at right angles to the foot, and with the condenser and objectives *in situ* it must remain absolutely firm, without any tendency to spin or topple over. A distinct pressure should be necessary to produce this result.

It should be remembered that the horseshoe foot is unsteady when in the horizontal position, even with the short tube common to the Continental microscope. It is utterly impossible if the long tube be required. There has not been one of the leading microscopists who has not insisted on the superiority of the tripod foot. As Dallinger pointed out, the Continental foot endeavours to secure stability by weight. Such an endeavour is wrong, and can be secured to a far greater degree and with far less weight by the tripod. Some persons have urged that the horseshoe foot gives freer access to the substage. This is a peculiar objection when one considers the very primitive substage arrangements supplied with the best of the Continental microscopes. Moreover, a very slight working experience of the tripod foot quickly demonstrates the fallacy of the argument, and this is recognised by all microscopists whose opinion carries any weight whatever. As Dallinger remarked, the principle of the horseshoe foot is mechanically wrong, and this argument remains as strong as ever to-day.

The stage E is for the support and manipulation of the slide. It is pierced centrally for illumination from the mirror or substage condenser. Two forms of stage are made, known respectively as the plain stage and the mechanical stage. The aperture of the plain stage should never be less than 1½ inches in diameter, and preferably more. These stages are usually supplied with two metal springs, as shown in fig. 23, to clamp the object; but if a sliding bar can be purchased, this is preferable, because it allows a more systematic search of the specimen.

A mechanical stage is of the greatest utility to the microscope user, but it is always preferable to purchase this as an integral part of the microscope. If this cannot be done, and a mechanical stage is to be added at a later date, select one that is rigidly made. Watson's Service Mechanical Stage, which can be adapted to other models, is the nearest approach we have seen to a built-in mechanical stage, and represents an almost ideal attachable microscope stage. The Spencer attachable stage is also excellent.

The attachment of the microscope stage is a most important point. Upon this depends whether through years of constant use the microscope stage will remain in correct position towards the optical axis, whether it will support weights such as the Petri's dish, etc. The attachment should be made by solid lugs projecting from the microscope limb for some distance under the stage, and through which the stage itself is bolted. Otherwise, with the attachment of a mechanical stage later, torsion is sure to result. Until recently the stage was treated by the majority of microscope designers without sufficient attention. Often, only slightly secured to the limb, it had to bear the weight of the spiral focussing screw or substage apparatus, the condenser and the mirror. When the fitting of many microscope stages is examined carefully, it will be wondered how these instruments endured such treatment.

The body tube c carries various lenses of the instrument. As previously stated, it should not be less than 35 mm. diameter, and in a research instrument it should be provided with a rackwork draw tube or an assurance obtained from the manufacturer that such a draw tube can be fitted. This is not a necessity in a students' instrument—a plain draw tube meets all cases, but these draw tubes must be divided into mm., so that the tube length can be instantly read. On purchasing, ascertain whether these engraved divisions are calculated to show the total tube length with or without the nosepiece, as this makes a difference of 12 or 17 mm.—sufficient with a good objective to ruin its performance.

In the past there have been three tube lengths, viz. the English 250 mm., the Continental 160 mm., and a compromise between the two of 200 mm. The English and Continental have been known as the long and short tubes respectively. Microscopists will find over a period of time that they do not wish to confine themselves to objectives by any single maker, as most makers have some lens in which they excel, and a draw tube is therefore necessary which will enable the user to obtain any tube length. At the present time all English lenses can be obtained for an 8-inch tube length, and therefore no microscope should be purchased unless it gives a range of tube length up to 210 mm., the spare 10 mm. often being necessary to correct for cover glass variations.

The firm of Zeiss, when designing their apochromatic objectives for the English microscope, took the mechanical tube length of 10 inches, or 250 mm., but this had already ceased to be the English tube length, which had been reduced to 200 mm., or 8¾ inches, this being obtainable on any well-constructed microscope. Fig. 24 shows method of measuring tube length.

The Continental mechanical tube length is 6½ inches, or 160 mm. If objectives were all accurately corrected for the tube length they are supposed to work upon, and would work equally well upon any thickness of cover glass, this would be satisfactory, but when corrections for cover glass have been made. Many Continental objectives actually require a tube length of 4½ to 5 inches, which absurdly short length is not obtainable on any modern instrument.

It would be a world-wide advantage if opticians and microscopists would sink their prejudices, and adopt the suggestion of Professor A. E. Conrady, to correct all their objectives for a mechanical tube length of 8 inches, or 200 mm. This would allow a fair margin for the correction of cover glass thickness by means of the draw tube. Unfortunately where objectives have been sent out corrected for 200 mm., they have often been used on a short tube;

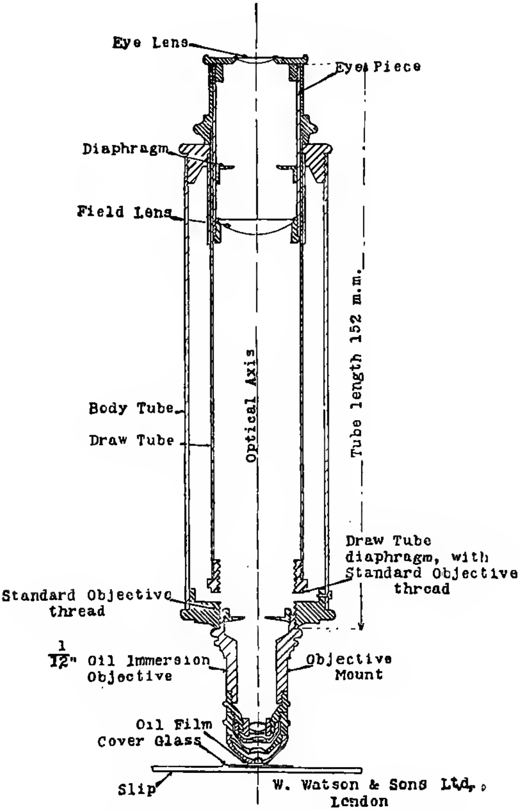
Eye Lens
Eye Piece
Diaphragm
Field Lens
Optical Axis
Tube length 152 m.m.
Body Tube
Draw Tube
Draw Tube diaphragm, with Standard Objective thread
Standard Objective thread
$\frac{1}{12}$" Oil Immersion Objective
Objective Mount
Oil Film
Cover Glass
Slip
W. Watson & Sons Ltd., London

FIG. 24.—Method of Measuring Tube Length

consequently they do not perform to the best advantage, neither do they develop their full magnification. They have then been placed in competition with Continental lenses corrected for 160 mm. Obviously no true comparison can be obtained, but frequently excellent objectives have suffered on this account.

Tube length will be referred to later when objectives are considered.

The bottom of the body tube is provided with a nosepiece D for carrying the objectives, and the eyepiece fits into the draw tube at the opposite end (fig. 23). This eyepiece should slide in easily, and not require pushing. The draw tube should be examined to see that at the opposite end of the eyepiece there is a diaphragm threaded with the standard R.M.S. screw. If it is not provided with this, reject the instrument, or insist upon the maker inserting it free of charge.

Unfortunately a still more glaring error is occasionally encountered. Some stands are offered without a diaphragm of any sort at the end of the draw tube, seriously harming the performance of the objective. On the other hand, there are microscopes offered, with the diaphragm of so narrow an opening as to prevent the full numerical aperture of the objectives being developed. Either of these faults is fundamental.

The next adjustment to consider is the rack and pinion coarse adjustment G. In the past stands have been made with a sliding coarse adjustment, but very few of this type are offered at the present time, and they should be resolutely avoided, as no good work can be done with them, and as soon as the owner begins to realise the demands he will make upon his microscope, the instrument will be discarded; it is therefore so much waste of money.

We have already referred to three points in connection with the coarse adjustment, (*a*) the range, being its ability to focus objectives from 2 inches (50 mm. focal length) to a $\frac{1}{12}$-inch oil immersion objective; (*b*) the smoothness required which should enable the user to focus a $\frac{1}{6}$-inch objective without use of the fine adjustment; and (*c*) proper attention to its fitting.

There is one other point. It occasionally happens that the rack and pinion do not properly correspond, in which case there arises what is known as a loss of time. This means that the milled head can be rotated for, sometimes as much as $\frac{1}{8}$th of a turn, without altering the focus in any way. This is not a common fault, but one that occasionally occurs, and should be guarded against.

The fine adjustment F is a movement of the microscope, which has created probably more controversy on the mechanical side, and is given a wider interpretation by the different designers than any other. There have been many forms of this in the past which have been discarded, and it can now be divided into three remaining types—the horizontal lever form, the direct acting micrometer screw, and the side lever pattern. The direct acting micrometer screw is now fitted by one or two Continental manufacturers, but only upon their cheapest stands, and it is the least satisfactory of the modern forms. The fine adjustment preferred by the writer is the lever movement designed and manufactured by Messrs. Watson & Sons, and shown on the instrument in fig. 23. The side form has had a wide vogue for the past ten years, owing to the supposed comfort in using it, but one should bear in mind that in the horizontal type of lever there is only one movement, viz. the up and down movement acting on the middle slide, while in the side fine adjustment there is also a side movement combined with the up and down movement of the body. To overcome this Messrs. Zeiss have embodied a clock train of wheels, shown in fig. 25. The result is to give the direct action provided by the horizontal lever pattern of fine adjustment, but how this clockwork gear will wear over a period of years is a matter yet to be determined.

A frank admission that the horizontal lever type of fine adjustment is the most durable and the most satisfactory form yet made would be of considerable assistance to all microscope manufacturers, substantially reducing the cost of production and rendering the instrument incomparably more efficient.

There is one other form of fine adjustment known as the nosepiece pattern, and this is figured (fig. 26) to show a model that should **in no circumstances whatever** be purchased. It is occasionally to be met with in second-hand shops, and is offered to medical students. With this no fineness nor steadiness of adjustment is obtainable, and it cannot be too strongly condemned.

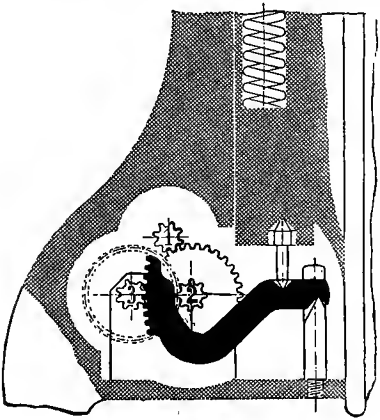

FIG. 25

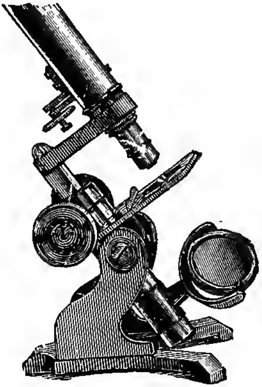

FIG. 26

**Tests for Fine Adjustment.**—Before finally purchasing a microscope, examine the fine adjustment to see that it works smoothly. The loss referred to in the coarse adjustment is unfortunately much more common in the fine adjustment, especially in the horizontal position. Obviously, if later the stand is to be used for photo-micrography, and there is any loss in the fine adjustment, the instrument will be useless. We have already given instructions on how to test this.

The other test is for lateral motion in the fine adjustment. For this purpose focus with a $\frac{1}{6}$-inch objective upon a diatom, or a bacterium. Then move the milled head in and out of focus steadily, and observe the image. If this shows any apparent movement from side to side of the field, the fine adjustment of that instrument should be immediately suspect. This test will be useless, however, unless before commencing it is ascertained that the light is accurately central and axial with the objective. The method for centration of the light through the condenser is given in a later chapter. Neglect of this precaution may cause a motion which is not due to the fine adjustment at all, or, on the other hand, it is just within the bounds of possibility that it will neutralise the error in the fine adjustment. I have seen cases where microscope and objectives have been condemned merely on account of incorrect centration of light. When this has been corrected, the performance has been such as to entirely alter the user's opinion of the instrument.

**The Mirror.**—The mirror N should be at least 40 mm. in diameter, plane on one side, and concave on the other, well fixed so that with normal use it is not likely to become loose in the gymbal. The majority of students' microscopes are fitted with mirrors far too small to be satisfactory, and if they are examined it will often be found that the mirror gives a variety of ghost images. Another important point is that the mounting of the mirror should be such that it would give it the power of throwing the light along the optical axis of the microscope, when inclined at an angle of 45°. If this is not done, a certain obliquity of light is always present, which does not enable the optical equipment of the microscope to perform to the best advantage. It would be of great advantage if all plane mirrors were mounted so as to admit of rotation in their cells, as troublesome double reflections can often be done away with by this means.

Passing reference has already been made to the substage s, a part of the microscope which is of the very highest importance, and no reliable work of any sort can be done without it. This is a movement which is entirely due to the English microscope designers and manufacturers. Its importance was appreciated so long ago as 1690, when Bonnani produced a horizontal microscope of which the principles were extremely advanced, and this had a focussing condenser. Most microscopes of the period until 1720 had some system of condensing lenses. It was then dropped until reintroduced in the nineteenth century, by the English firms who then had the supremacy in mechanical microscope design which they have ever since retained.

The necessity for a condenser was appreciated by microscope workers throughout the world, and Continental firms were forced to introduce it. But it is only within recent years that they have introduced achromatic substage condensers and centring screws. In spite of this, after careful examination of all forms, there is in the opinion of the writer not one good or efficient substage made by any Continental maker at the present time. The centring fittings are invariably sliding sleeves, not integral parts of the substage, and the English model embodying these, with the swing-out motion, is unquestionably superior.

In the cheapest students' instruments a substage is not provided. They are most frequently fitted with what is known as an understage. This consists essentially of a spring tube fitting, in which the condenser slides, and allows a certain amount of latitude for focussing. This tube is screwed in the best types to an independent arm, allowing the understage fitting to be swung out of the optical axis. In other forms it is merely fixed to the under surface of the stage. A better form is known as the spiral focussing screw (fig. 27). Here the arm is focussed up and down by means of a spiral screw which has a jacket or sleeve to which the arm is attached. It focusses along a guide. This provides a much more delicate focussing movement, and will be found more convenient, especially as its price is low. It is to be deplored that an attachment of this sort, which is in use by the majority of students, has not had more care in the rigidity of its design. In most cases it is fastened to the under surface of the stage, and the spiral focussing screw itself has no bottom supports. The effect of a blow, therefore, will probably be to throw the whole attachment out of the optical axis.

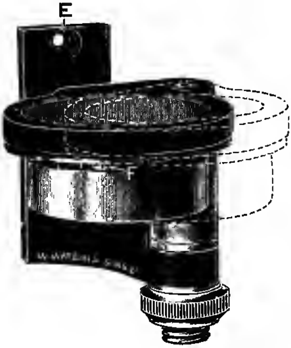

Fig. 27.—Spiral Focussing Screw

We have seen a student's microscope in which the limb is continued below the stage, and on which the understage motions are mounted, and this gives promise of a far greater working efficiency and durability. Another improvement would be the provision of centring screws to the spiral focussing attachment; but if really serious work is intended, too much emphasis cannot be laid upon the necessity of a properly made substage with diagonal rackwork focussing and screw centring. Such a substage is shown in fig. 28. In the highest class of research instrument, a fine adjustment is provided for the substage, shown on the instrument in fig. 23. There is a difference of opinion as to the necessity for this extra, but I think it will be found very convenient to the critical worker, although it is not an essential.

The last point in substages is that they are heavy fitments, and it is advisable that they be mounted not on the stage, but upon the limb itself. This has been done in the past on the best instruments by three microscope makers, and I hope it will become general, not only on the best instruments, but on students' instruments, as with the weight of the substage dependent from the stage there is not only the liability of torsion through this additional weight, but it is impossible to focus the substage to get the best result, and to retain the object in focus, the weight of the hand on the substage milled head being sufficient to throw the object out of the focal plane.

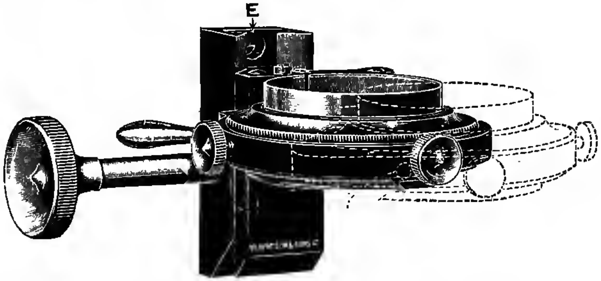

FIG. 28.—The Best Type of Compound Substage

**Objectives.**—Of recent years objectives have undergone a vast change for the better, particularly in the case of those designed for the use of students. In fact, the improvement in the ordinary achromatic series has been so great, that those not intimately acquainted with critical microscopy might reasonably be excused were they to mistake them for apochromatic objectives, especially if used with a green screen. Their performance is such as to well merit the title of semi-apochromatic objectives. The objective, the condenser, and the eyepiece are three of the most important items on a microscope, and of these the most important is the objective. Great care, therefore, must be exercised in its selection, and none should be purchased unless they bear the name of a maker who is actually manufacturing at the present time.

Good accessory apparatus may occasionally be met with second-hand, and this is particularly the case with objectives, but great care must be exercised in purchasing. No lens, new or second-hand, should be bought unless it bears the name of a recognised maker, and then only after careful testing. The safest plan is to buy second-hand accessories from a manufacturer only, as they have to take microscopes in part exchange for new instruments, and are therefore able to sell the second-hand ones at lower prices than if it were their main business. Further, they have facilities for adjusting and repairing all they sell. Real bargains are often to be found at both Messrs. Charles Baker, and W. Watson & Sons, of High Holborn. Not only do these firms give every opportunity for testing instruments and lenses on their premises, but they are willing to allow goods to be taken away on approval, which is always essential.

Most students' microscopes are listed complete with objectives. These objectives are usually paired up, 1 inch and $\frac{1}{4}$ inch, and $\frac{2}{3}$ inch and $\frac{1}{6}$ inch, and will cover all the beginner requires. Such objectives, if by recognised makers, are of an all-round utility.

A perusal of catalogues will show that sets composed of a microscope with case, eyepieces, objectives, etc., are substantially reduced, compared with the costs of the items purchased individually, the reason being that the actual microscope itself is by no means a profitable instrument to manufacture; so much is this the case that until the war, some foreign manufacturers refused to sell a microscope and case without any optical equipment.

**Qualities of Objectives.**—Workers in different branches of microscopy require varying elements to compose their batteries of microscopic objectives, and the types of objectives, even of the same focal length, vary considerably

Some points looked for by the histological worker in a high-power objective are entirely inconsistent with those called for by the microscopist who indulges in the pleasure of diatom resolution. The resolution of diatoms is an art in itself, and the microscopist who can successfully resolve the ultimate structure of these minute siliceous shells understands better than many microscope users the necessity of good equipment, properly set up and working to its best advantage.

To take the varying qualities in detail:

**Definition.**—The first point to look at in any microscope objective is its power of giving a clean-cut image of the specimen examined, as without this property the objective is utterly useless. This power in turn is dependent upon the eyepiece, the source of light, and its adjustment. For ordinary purposes students' lenses of standard quality should show no breaking down whatsoever with a ×10 eyepiece. Should this occur, it can be taken for granted that the objective is a poor one, and should not be purchased.

Some achromatic objectives show breaking down with higher power eyepieces than this, but there are makers whose objectives are both distinctly better in composition and manufacture, giving excellent performance with eyepieces of as high a power as ×27, while an apochromatic objective 8 mm. focal length which has been introduced since the war by an English firm, performs successfully with an eyepiece ×40. To use these higher power eyepieces to advantage, the utmost care must be exercised in adjusting the outfit. The tests applied for definition will be described at the end of this chapter.

**Numerical Aperture.**—A description of this has already been given, and the power to resolve fine structures is dependent upon the numerical aperture of the objective, and can be obtained by no amount of empty magnification. In fact, there are oil immersion objectives of $\frac{1}{7}$-inch focus, in common use to-day, which with a ×10 eyepiece will actually show considerably more structure than a dry $\frac{1}{10}$ inch or even a $\frac{1}{12}$ inch, of which the numerical aperture is not so high, although there is a greater magnification.

Apart from the lack of apochromatic correction, and high aperture, microscope lenses offered up to twenty-five years ago were great offenders, and extreme caution should be exercised in purchasing old objectives, unless the seller is willing to give a written guarantee of the numerical aperture and initial magnification. Effective numerical aperture itself is dependent upon the correct adjustment of the condenser, while the work to be done will necessitate various numerical apertures in the different lenses used.

Thus the microscopist who wishes to resolve diatom markings, the cytologist, and the research worker should all purchase the highest numerical apertures obtainable, bearing in mind that, as we have said earlier, aperture is dependent upon the condenser.

The microscopist who purchases an apochromatic objective with a numerical aperture of 1·37, and uses it with an ordinary uncorrected Abbe illuminator, which, although it may have a numerical aperture of 1, transmits an aplanatic cone not exceeding 0·50, is, assuming he uses his condenser and objective to the best advantage, only going to obtain an effective numerical aperture of $0{\cdot}93 \frac{(1{\cdot}37 + 0{\cdot}50)}{2} = 0{\cdot}93$.

For the work of the student moderate apertures will fulfil all his requirements, and the normal numerical apertures of the objectives are given below.

There are firms who offer objectives at lower prices and of lower numerical aperture, but valuing them on the formula of the optical index it will be seen that they are not a bargain. For the amateur in general, if he is a beginner, I should strongly advise him to commence with moderate apertures, to master thoroughly the use of his objectives, the technique of the microscope, and then decide upon the purchase of objectives of higher aperture, should the work require them.

**Working Distance.**—It has already been stated that the designations of objectives in focal length, whether in inches or in mm., do not represent their actual working distance, which is always substantially less than their marked focal length. The higher the numerical aperture, the shorter the working distance, as obviously a wide angle cannot be subtended at more than a given distance from the slide, and this is another reason why students should commence by purchasing objectives of moderate numerical aperture, especially as thick cover glasses are used by the great majority of students. Objectives of high numerical aperture or short working distance will not work through thick cover glasses. Later, if blood work and general diagnostic work is commenced Hæmacytometers are used, which have a cover glass with a thickness somewhere in the order of 0·5 mm. As a guide the following list of objectives and their numerical apertures will be found generally useful. Objectives should not be purchased of a lower aperture than this for the stated focal length, which has been calculated to give the most useful all-round value on the optical index formula for students' lenses:

$\frac{1}{2}$-inch objective n.a. 0·35.
$\frac{2}{3}$ ,, ,, ,, 0·25.
$\frac{1}{6}$ ,, ,, ,, 0·74.
$\frac{1}{4}$ ,, ,, ,, 0·65.
$\frac{1}{12}$ ,, (oil immersion) objective n.a. 1·25.

**Flatness of Field.**—Theoretically, this means that if a truly flat object is observed, of which all the structure lies in one plane, the image formed by the objective and examined by the eyepiece should be in the same focal plane over the whole surface of the field. Actually, it may be categorically stated that the better the quality of the objective, the less flat the field. Consider for a moment exactly what the word 'focus' implies. It is the theoretical point at which all the rays passing through the optical system composing the objective meet, in such a manner that a perfect image is obtained. Obviously, if more than a point is put in focus, the correction cannot be so good, and it must be inside the best focal point of the objective. In the past flatness of field has been the nightmare of the conscientious computor who wants to give the user the best lens of its type possible consistent with the price it was desired to pay. Such lenses naturally would not have a very flat field, with the result that they were not appreciated, and were withdrawn.

With low-power objectives the lack of flatness of field is not very apparent, when ordinary achromatic series are under consideration. The 2 to $\frac{2}{3}$ inch objectives come nearest to the ideal, and have usually a flatter field than any other; but immediately higher power objectives are used, it will be found that, for reasons stated, the more perfectly they are corrected, the more curved is the field, and this is absolutely unavoidable.

**Apochromatic and Achromatic Objectives.**—Apochromatic objectives, if adjusted at the correct tube length, correspond with their computation when the correct cover glass is used. If also the correct eyepiece is employed it should show no trace of colour, if such objects as diatoms or similar specimens are examined.

The most that should be seen is a faint trace of tertiary spectrum. Achromatic and semi-apochromatic objectives when submitted to this test will discover the secondary spectrum when colourless objects, or those showing a very slight trace of colour, are examined. Stained preparations are perfectly useless, when testing a microscope objective, to acertain the quality of its colour correction, unless the lens under examination is of such extremely poor quality that the chromatic aberration dominates the colour of the stain.

In practice both the Abbe test plate and the diatom are employed.

**Apochromatic Objectives.**—These are so high in price that few readers of this book are likely to purchase them, and I think it highly probable that to the ordinary student they are of less value than good achromatic objectives. Even pre-war apochromatic objectives were the most expensive obtainable, and the present-day prices show approximately a 15 per cent. increase over pre-war prices.

These objectives are much more difficult to work with than the achromatic type, as they are extremely sensitive to alteration in tube length, thickness of cover glass, etc., points which are not quite so important in the achromatic series. For the best and highest results capable of being produced by a microscope, they stand unrivalled, and there is no system as yet worked out, which will allow finer detail to be seen with sufficient accuracy to form a correct interpretation of the image, than a properly set-up microscope with apochromatic objectives working at full aperture. The research worker invariably has apochromatic objectives in his battery.

The introduction of apochromatic objectives is the result of the work carried out by Professor Abbe and Messrs. Zeiss. Shortly afterwards Powell & Lealand, of this country, followed their lead, and produced objectives of remarkably fine performance, but I do not think that they ever produced a real apochromatic. All the lenses engraved as apochromatics that I have seen emanating from this firm still show some outstanding colour, which cannot be eliminated, and this remark I think applies to all apochromatics made by English firms until quite recently. Since the war, however, Messrs. W. Watson & Sons have made a series of apochromatic objectives from the computations of Professor A. E.

Conrady. These will compare more than favourably with any that have ever been produced in this or other countries. They will be referred to again, but here it may be said that in the opinion of the writer they are the first strict apochromatics an English maker has ever produced.

The present series comprises four objectives:

16 mm. n.a. 0·30.
8 ,, ,, 0·65.
4 ,, ,, 0·85.
2 ,, ,, 1·37 (oil immersion).

I have examined many of these lenses, and found them of wonderful uniformity. The 4 mm. is, I think, the finest high-power dry lens yet made, whilst the 2 mm. is the best oil immersion objective of that focal length I have yet met with. It is interesting to note that it will focus through a No. 2 cover glass. None of these objectives are below the stated numerical apertures, the n.a. being guaranteed by the makers.

Apochromatic objectives are also made by Reichert and Leitz. Most of them are of high quality. The advantages of these lenses lie, in the first place, in their absence of colour on critical tests. In the second place, in their very high numerical apertures, which give in consequence a very much more brilliant image, and far higher power of resolution. In the third place, a good apochromatic objective is capable of standing high-power eyepieces. This is a most important point, and cannot be too strongly emphasised.

**Testing Objectives.**—It is quite impossible for the novice to test objectives, as, to master the methods, considerable experience is required, and the personal factor is also a very important point.

The following paragraphs outline a series of simple tests for objectives, commonly required by the student, and will enable him to form a fair judgment of their capabilities. It will be noticed that these paragraphs are subdivided for objectives of low power, medium power, and high power, including oil immersion objectives.

**Low-power Objectives.**—This includes objectives having focal lengths ranging from 2 inches up to $\frac{1}{3}$ inch (8 mm.). They should all bear a full cone of illumination. By this is meant that the condenser should be accurately focussed so that on removing the eyepiece and examining the back lens of the objective, this back lens will be seen to be filled with light. To test this, move the diaphragm of the condenser, watching the back lens. There is a point where the closing of the diaphragm will begin to cut off light from the back lens of the objective, and the point just before this is a full cone.

Two test objects will suffice to form a fair judgment on the subject of the performance of low-power objectives: a large diatom such as (*a*) *Arachnoidiscus Japonicus*, or a slide of a *Polycystina*; (*b*) the proboscis of a blowfly. With the condenser in position and focussed, remove the eyepiece to see that the back lens is filled with light; replace the eyepiece, close the diaphragm, and focus the aerial image of the diaphragm, care being taken that this is central. Open the diaphragm until the back lens of the objective is filled with light, and focus the diatom. The image should be clear cut, and there should be no appearance of fog. Insert in the stop carrier of the condenser a medium-sized stop, and examine the diatom with the dark ground illumination thus obtained. If the lens is a good one, the diatom or polycystina should shine with a silvery whiteness, and there should be no trace of fog. On cheap quality objectives a lack of clearness of definition is perceptible, and such objectives should be viewed with suspicion. It will be well to examine these tests with a high-power eyepiece, say ×12 or ×15; the lens, if a good one, should show no trace of woolliness. The test of the blowfly proboscis lies in the fine hairs on the membrane between the pseudo-thoracic spiracles, which should show sharp as needle points with no apparent doubling.

The condenser should be focussed to give critical illumination, and the point of the fine hair covering the membrane examined. With a high-power eyepiece (not less than ×10) these hairs should show perfectly sharp points, clearly outlined. A poor quality of objective will show them split, and an apparent doubling of the ends. If a ×20 eyepiece can be obtained for this test, it is best to order it. In both cases the image of the object should be examined for colour fringes, and if these are at all pronounced the lens should be rejected.

**Testing Medium-power Objectives.**—This includes all dry objectives from $\frac{1}{3}$ inch (8 mm.) upwards, and with numerical apertures varying to 0·95. For this purpose five slides are required: (1) The *Podura* scale; (2) A specimen of *Pleurosigma Angulatum*; (3) *Pleurosigma Formosum*; (4) *Navicula Rhomboides*. These should be mounted dry, in styrax or in monobromide of naphthalene. (5) A slide showing the growing point at the root tip of an onion. This should be double stained to show the mitochondria.

First let us consider the *Pleurosigma Angulatum*. This should be examined with critical illumination, viz. the radiant should lie in the same focal plane as the objective, or in other words, should be focussed on to the object upon which the objective is in turn focussed. Remove the eyepiece, and examine the back lens of the objective. Close down the condenser diaphragm to about one-quarter of the diameter of the back lens of the objective. The central beam should then be seen surrounded by six diffraction spectra (fig. 29). Increase the aperture of the condenser until the edges of the central beam just touch the edges of the diffracted beams. Replace the eyepiece, which should be either ×12 to ×20, and with a slight alteration of focus, by means of the fine adjustment, either a clearly defined sharp white hexagon or clean black dots should be visible on the diatom. The black dots should appear black, and not greyish, and the better the objective the higher power eyepiece it will stand, showing these dots without any appearance of woolliness. In this, and in ensuing tests, particular care must be taken to adjust the condenser accurately, otherwise an objective may be condemned when the fault lies with the condenser.

A subsidiary point is that in fixing the illumination for the black and white dots any faults in the fine adjustment, viz. shake or slowness of motion, will become instantly apparent, as on this test the change should be instantaneous between the two images, and not require more than a $\frac{1}{10}$th of a revolution of the milled head of the fine adjustment. If there is any stickiness, the change over of the images will be delayed and jerky, while if there is any shake the image will appear to move from side side to side of the field.

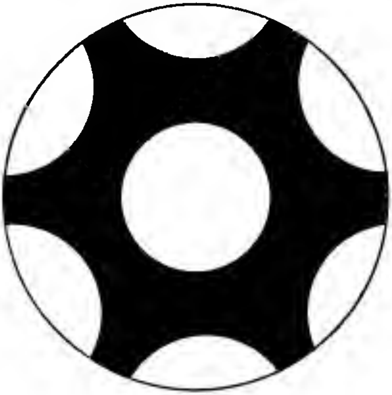

**Fig. 29.—Back Lens of Objective when Pleurosigma Angulatum is resolved, showing the Diffraction Spectra and the Central Dioptric Beam**

Under these conditions, but with a three-quarter cone of illumination, examine *Pleurosigma Formosum.* This valve forms a splendid test for chromatic aberration. The markings should show as distinct white balls, or dark black holes, as clearly as though they had been cut out from paper. I have recently examined a set of objectives offered by a German maker, which when tried with this test showed distinct red and green holes more like a paget-colour plate. These lenses, I am informed, are being sold in large quantities in this country, and were they tried upon this test I doubt if one would be passed.

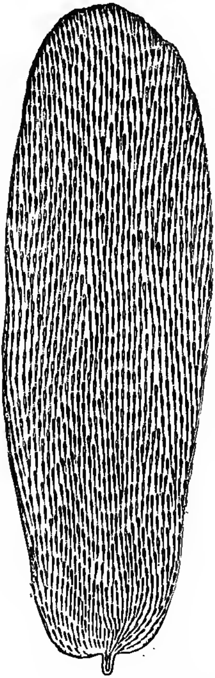

FIG. 30.—Test Podura Scale

*Navicula Rhomboides* is the next object to be tested. This is a test for aperture. The condenser should be set at $\frac{3}{4}$ cone, viz. passing a beam of light fully $\frac{3}{4}$ of the back lens of the objective, or if anything rather more. An eyepiece ×15 or ×20 should then be inserted, and the specimen should instantly show distinct white dots without any appearance of woolliness. The *Podura* scale (fig. 30) is still one of the best tests for the definition of an objective, despite the fact that it was introduced forty years ago. With this a good objective will perform at nearly full aperture. A high eyepiece ×15 or ×20 should again be employed, and the scale should be seen to be covered with distinct exclamation marks, as in the figure, each exclamation mark having at the thick end a distinct centre hole. The test lies in the power of the objective to show these markings distinctly, as clear-cut exclamation marks without any doubling of the pointed end, and at the same time showing the white mark in the centre clearly. The image should be without any trace of woolliness or breaking down in definition. A really good objective will stand a ×27 eyepiece without any breaking down, if the condenser be correctly adjusted.

The last test for medium-power objectives is the root of an onion, showing *Mitochondria* stained preferably by the iron-hæmatoxylin method. This specimen should be observed with at least a $\frac{3}{4}$ cone, and great care must be taken in adjusting the condenser for centrality and tube length, to correct for cover glass thickness. *Mitochondria* should present a sharp, clearly defined image, and go crisply in and out of focus in moving the fine adjustment milled head. This test is best made with an eyepiece of × 15, although a good lens should, as before stated, give good definition with an eyepiece ×20 or ×27. The frontispiece illustrates this test object, double-stained in red and black.

**Testing High-power Oil Immersion Objectives.**—These comprise all lenses from the $\frac{1}{7}$-inch oil immersion objective to the $\frac{1}{15}$-inch objective, and apertures varying from 1·20 to 1·40. The majority of the tests described for medium-power dry lenses are applicable to oil immersion objectives, but my own preference is for an iron hæmatoxylin stained specimen showing Mitochondria, a specimen of Trypanosomes stained with Giemsa, and Pleurosigma Angulatum. The tests with the specimen of Mitochondria and of Pleurosigma have already been described. With a specimen of Trypanosoma Lewisi, an accurate opinion of the tube length correction of the objective can be formed to within 5 mm. The condenser is centred, and the objective focussed upon the parasite, at one end of which will be found the tropho nucleus. If the objective is a good one, and correction for cover glass accurate, it should be possible to count the chromidia in the nucleus. Variation of tube length by 5 mm. on either side is sufficient to spoil definition. This is also an excellent test for responsiveness of the fine adjustment, and to ascertain that it is free from shake.

There is one other test which cannot be altogether recommended to the student owing to its difficulty, but which, if correctly applied, will unerringly differentiate between good and bad oil immersion objectives. This test is a favourite one of mine and also of Commander Ainslie. Take a slide of Amphipleura Pellucida,[1] and with an achromatic oil immersion condenser centre the light accurately. The specimen should then be focussed with a $\frac{1}{12}$-inch objective in position, and a × 20 eyepiece. The striation of the valve should be instantly apparent, although not resolved into dots. An oil immersion objective which will do this can be placed in the first class. If it will not answer this test, it might definitely be considered as a second-rate objective.

It cannot be expected that this striation can be as clearly marked as in the figures, as for both these purpose oblique illumination is necessary. With oil immersion objectives, colour correction can be tested by the examination of a specimen of Navicula Lyra. When examining with truly apochromatic objectives the dots will be seen standing up distinctly, and only black and white images are apparent, but with other lenses, a trace of faint apple-green and blue-purple is seen on the borders. Slides of bacteria, such as bacillus diphtheriæ stained by Neisser's method, are also good tests. In the latter, the polar bodies should show up clearly defined and crisply. In all these tests do not fail to observe the performance of the fine adjustment.

One peculiar point emerges from this, and it will be noticed by any one who tests his objectives carefully. Objectives that will perform well on histological and bacteriological specimens will not always perform equally well upon specimens of diatomacæ, but an objective which gives *really* critical definition of diatomacæ, will generally not fail to give a brilliant picture with bacteria and other specimens.

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[1] Preferably mounted in either monobromide of naphthalene, or better, in Realgar.

The reason for this lies in the correction. A lens corrected, to be free from chromatic and spherical aberration, and actually made to perform these functions, will give a colourless clear image of diatomacæ, and a true sharply defined image with bacteria. If, on the other hand, it is corrected purely for histological and bacteriological subjects, some computors leave a certain amount of residual colour, so as to cause the image of the specimen to stand out more boldly in relief, such lenses often being wrongly thought superior.

Such objectives on diatomacæ will give a very poor performance, and for this reason I would emphasise the importance of testing on diatoms as well as upon other specimens.

The objectives that will be found of most use to the beginner or student are the 2 inch, 1 inch, $\frac{1}{2}$ inch, and either the $\frac{1}{4}$ inch or $\frac{1}{6}$ inch, but for most purposes the $\frac{1}{6}$ inch will be found better than the $\frac{1}{4}$ inch. As I have before remarked, although makers may excel in the high quality of their objectives, they may have in each series some lens of which the outstanding qualities overshadow those of other makers. In other cases, a maker may supply a set of lenses of ordinary quality, but have one or two which are worth purchasing.

I cannot too strongly emphasise the necessity of purchasing a microscope direct from the maker himself. You will then be certain that the instrument has been through his inspection room, has had the objectives tested, and has been sent direct to you. Also that all the accompanying accessories have come from this maker, and he will take responsibility for them.

## MICROSCOPE STANDS

In 1855 the Society of Arts offered a prize for the best microscope that could be bought for three guineas, which was won by Messrs. Field & Co., of Birmingham. This pattern has been made by many opticians since, and is known as the Society of Arts Microscope. It is shown in fig. 31.

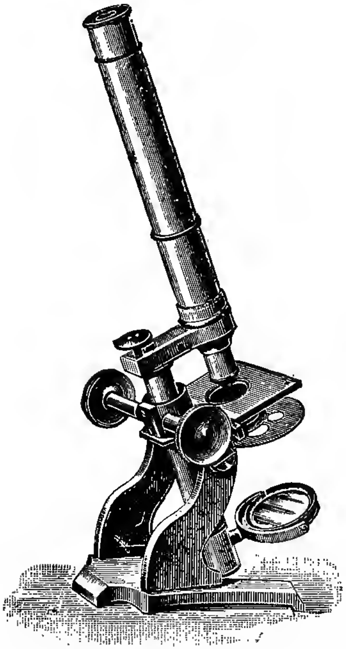


FIG. 31.—Society of Arts Microscope

The instrument as a rule is sold in case with one eyepiece and a dividing objective making 1-inch, $\frac{1}{2}$-inch, and $\frac{1}{4}$-inch powers. If no better can possibly be afforded, it will serve to introduce the student to the world of the minute, but for serious work it is totally unsuitable, and we hope readers of this little book will endeavour to obtain something better.

We will assume that real work combined with pleasure is intended from the first, but that little more can be afforded; what is the best that can be done? Failing a second-hand instrument, which may often be picked up cheap from Messrs. Baker & Son or Messrs. Watson.

A new model, the "Kima" of Watson, will meet the case. This microscope is illustrated in fig. 32. It is a thoroughly well-made and good-looking instrument, the workmanship and finish being of the highest class. It has a fine adjustment, the coarse adjustment being of the first-class, and will focus a $\frac{1}{4}$-inch or $\frac{1}{12}$-inch objective satisfactorily. The cost in fitted oak case is £7. For another 25*s.* an understage capable of focussing a condenser can be added, which greatly increases the utility of the instrument. This

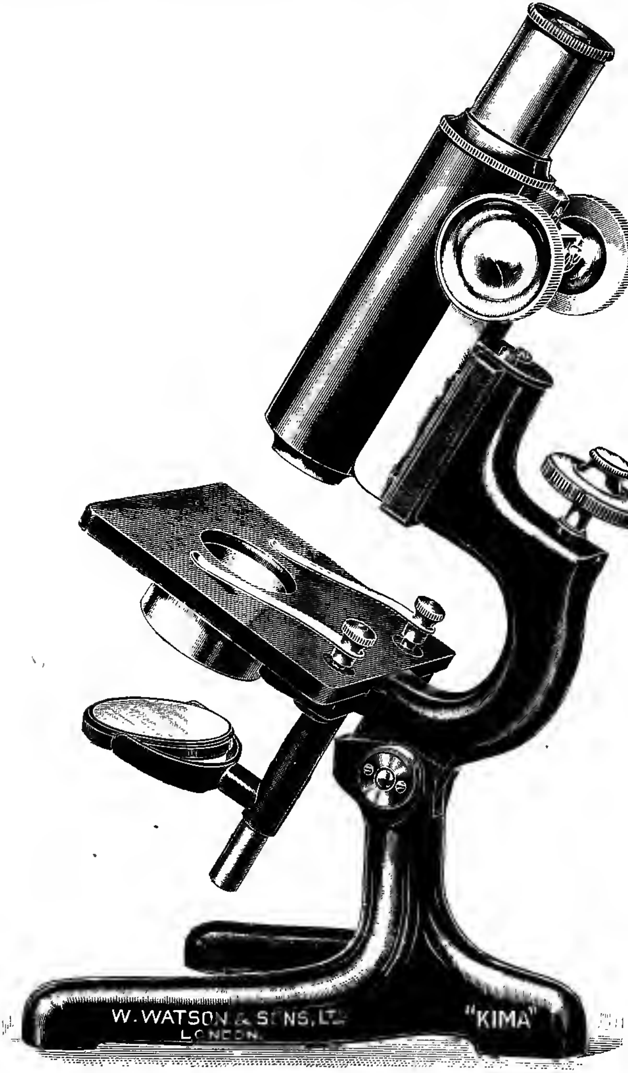


FIG. 32.—Watson's 'Kima'

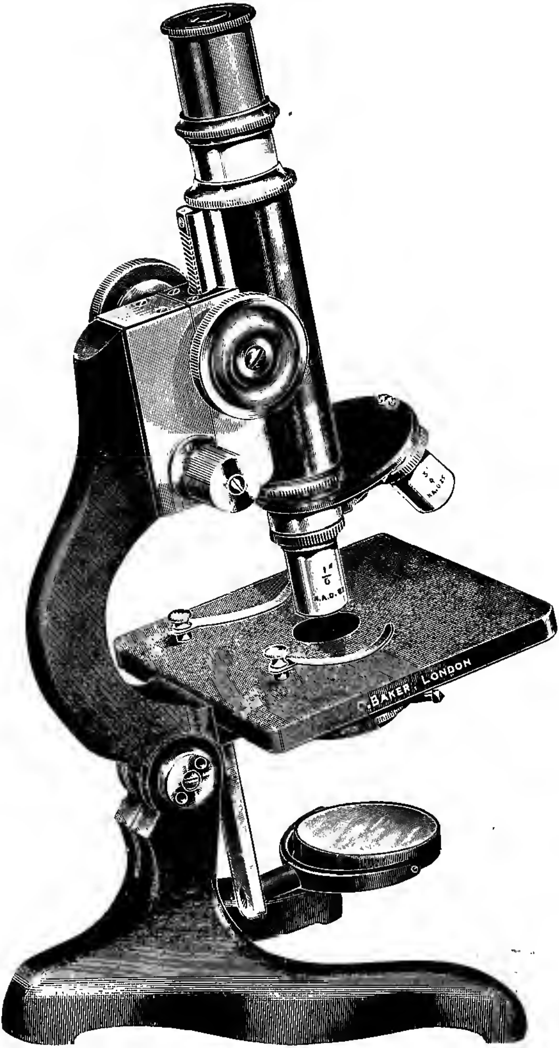


FIG. 33.—Baker's Student's Microscope

model has a lever fine adjustment, standardised fittings for addition of focussing understage, and mechanical stage. The price with eyepiece, $\frac{2}{3}$-inch and $\frac{1}{6}$-inch objectives is £11 10*s.*, representing most exceptional value.

Let us next consider more advanced models.

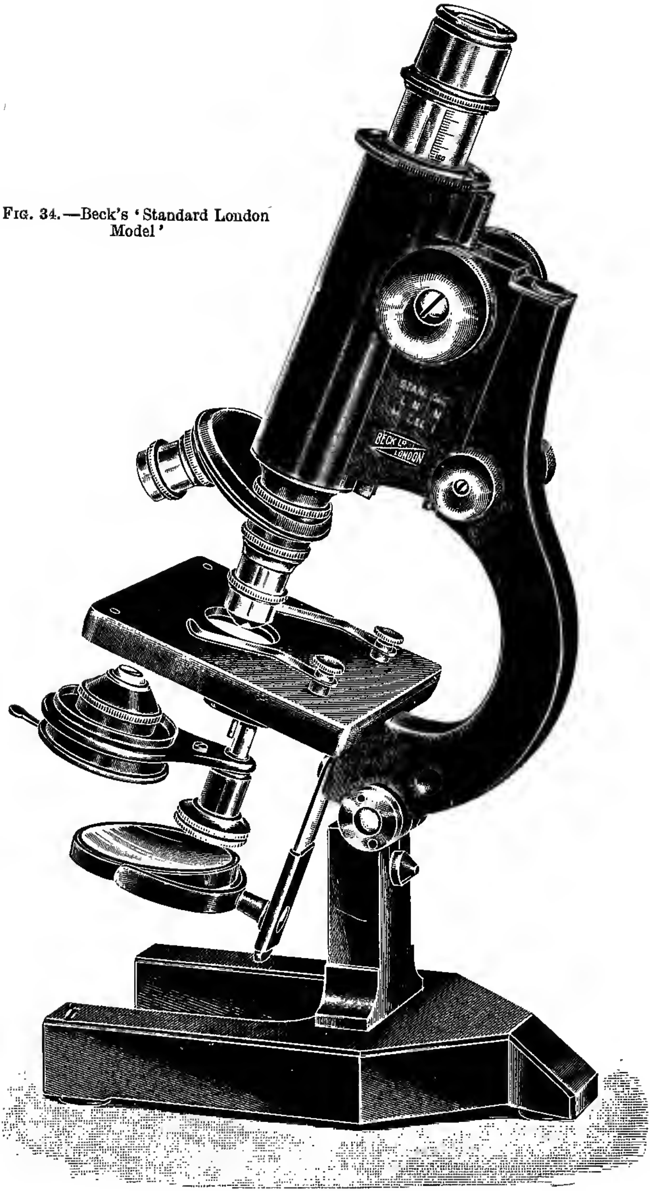
Fig. 33 shows Messrs. Charles Baker's student's microscope. The foot is a modified horseshoe pattern which will be found steadier than the majority of the strict Continental feet. It is supplied with a reliable fine adjustment working from the side, and a screw focussing understage capable of carrying an Abbe condenser. This stand in case without optical parts will cost £11.

Messrs. Beck make a very similar instrument at the same price. It is known as the 'Standard London Model' and is shown in fig. 34. This microscope was designed to fulfil the specification prepared by the British Science Guild for a standard microscope. The aim here has been to provide the student with a simple stand capable of being added to as necessity dictates. Thus the stand may be purchased for £11 in the first case, and is provided with coarse and fine adjustments and a simple understage fitting. Later, when the student feels the need of higher class condensers and a mechanical stage, these can be fitted by himself with no other tool than a screw driver. This is a very desirable feature in students' microscopes, and a feature that will only be found in English instruments.

Messrs. Watson make a still better instrument illustrated in fig. 35. This microscope is known as the 'Service,' and for general students' use is probably the best instrument made. It may be purchased with a plain understage fitting for £10. A special compound rackwork substage with centring screws is made for this instrument, and may be attached by the worker himself. Such an addition makes a really fine instrument capable of the most serious work.

Every worker who really means to pursue microscopic

FIG. 34.—Beck's 'Standard London Model'



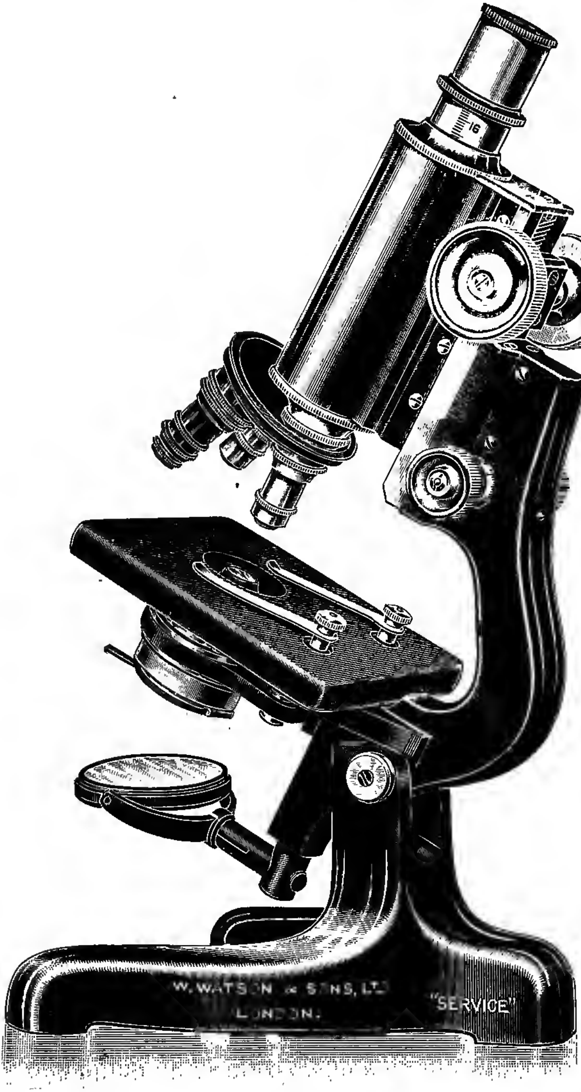


FIG. 35.—Watson's 'Service'

work of any sort is again strongly advised to purchase if possible an instrument with a properly fitted compound substage with centring screws. Let us, therefore, next see what instruments can be had of this class. Fig. 36 shows Messrs. Swift's standard microscope; it is a somewhat similar model to the Beck standard instrument, and may be had in case with compound centring substage for some £15. The Service with substage is of this class.

The finest example of this class of instrument is in our opinion the Edinburgh students' Model F of Messrs. Watson & Sons. The instrument is illustrated in fig. 37, and costs in case with compound rackwork and centring substage some £20. For excellence of design and high-class workmanship this instrument is unsurpassed. Nothing approaching such an instrument can be obtained on the Continent for students' use.

Fig. 38 shows Messrs. Swift's 'Army' microscope, which was designed to meet the requirements of Sir A. E. Wright. The workmanship is excellent, the price being £30.

The Continental firms, Leitz and Zeiss, also supply students' instruments of this class, but without efficient substages. Messrs. Leitz, however, are now offering an excellent stand provided with substage as made in England and with a tripod foot. The stand is illustrated in fig. 39 and is of excellent workmanship. Although we have expressed very definite opinions regarding the Continental type of stand, we must make an exception in many ways with the stands of Messrs. Leitz, represented by Messrs. Ogilvy in this country. The feet, wherever the Continental pattern is used, really amount to a modified type of tripod and are remarkably steady. The workmanship is of extraordinary excellence, and Messrs. Ogilvy can supply them with an English type of substage with centring screws. The interchangeable monocular-binocular stand is, we consider, the finest of its kind yet placed on the market, whilst

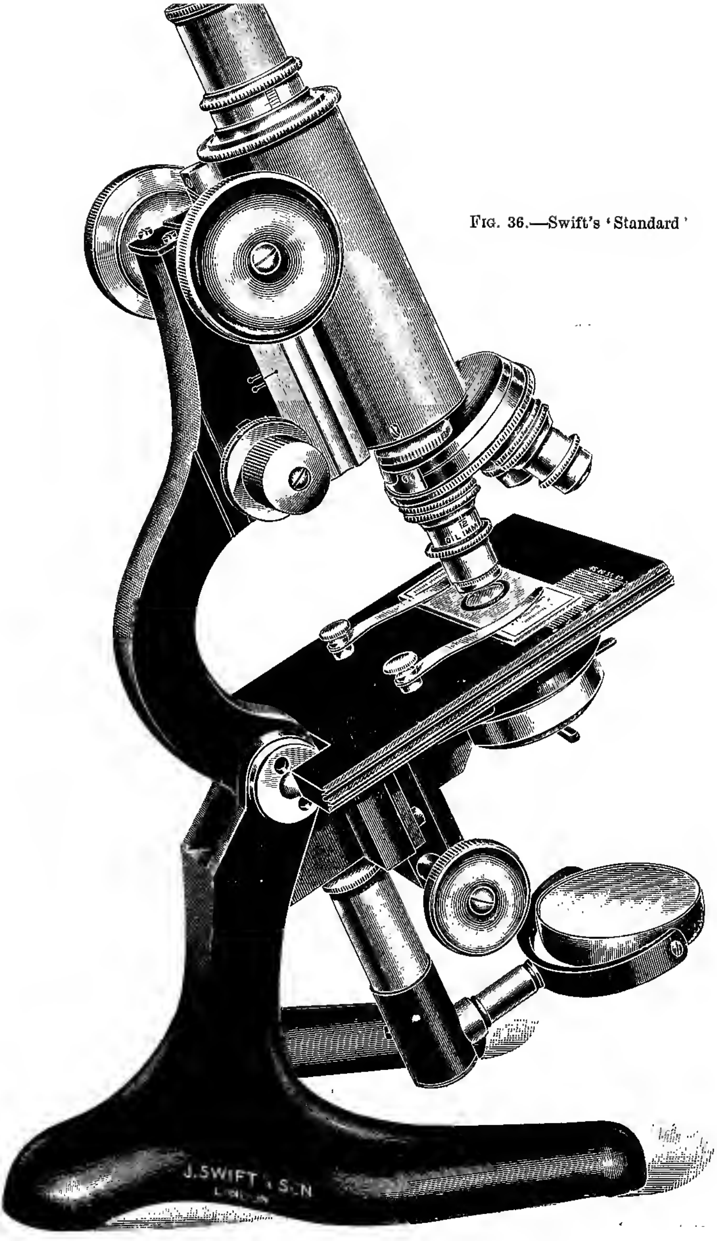


FIG. 36.—Swift's 'Standard'

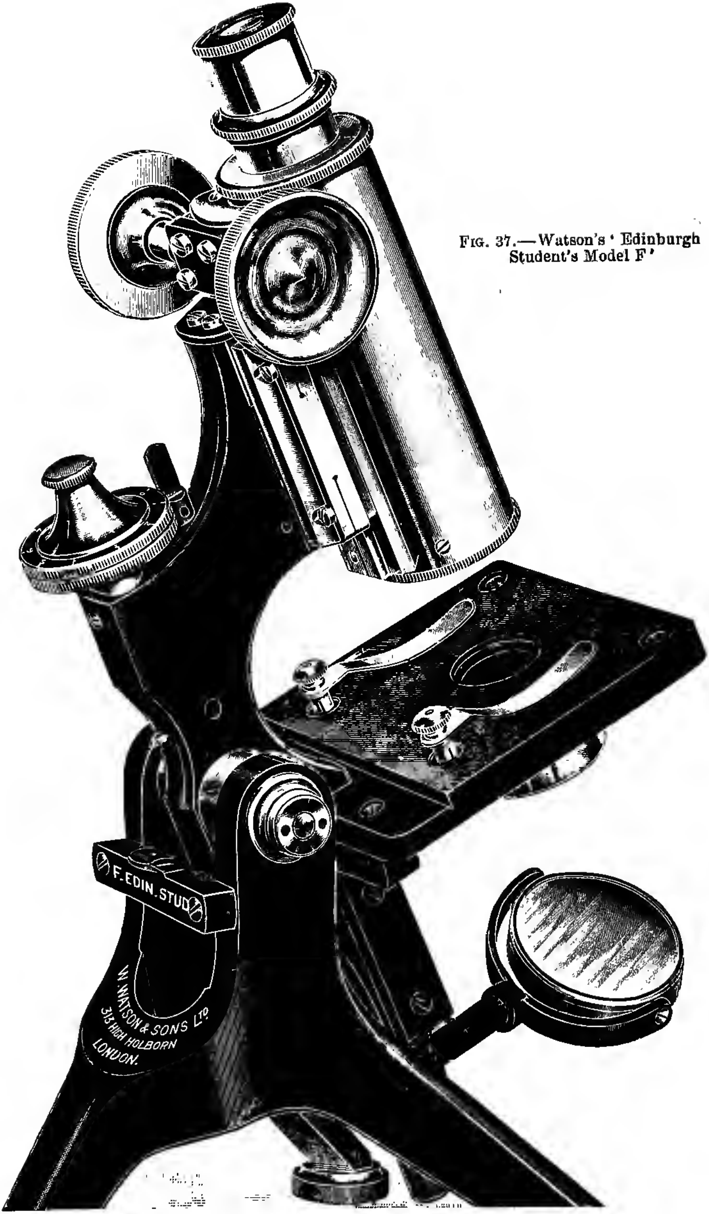


FIG. 37.—Watson's 'Edinburgh Student's Model F'

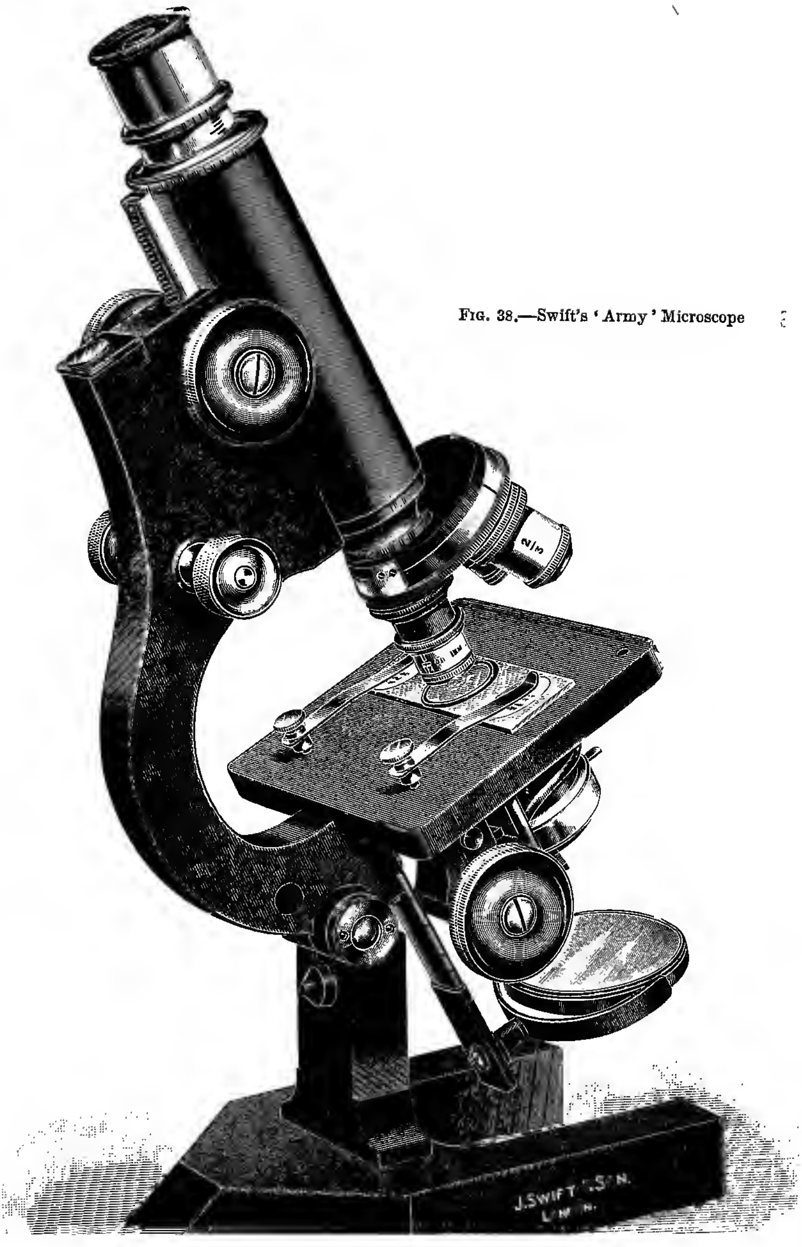


FIG. 38.—Swift's 'Army' Microscope

the tripod pattern student's instrument is of the highest excellence.

The new electric lamp recently brought out by Mr. Ogilvy is, we think, one of the best types of illuminant yet supplied to the ordinary worker. Messrs. Leitz have designed a new series of lenses for their student's stands which are of the semi-apochromatic variety, and without exception they will be found of great excellence. A second series known as the fluorite lenses represent the very highest types of achromatics; indeed, they are difficult to distinguish from the true apochromats. Messrs. Zeiss's stand is shown in fig. 40.

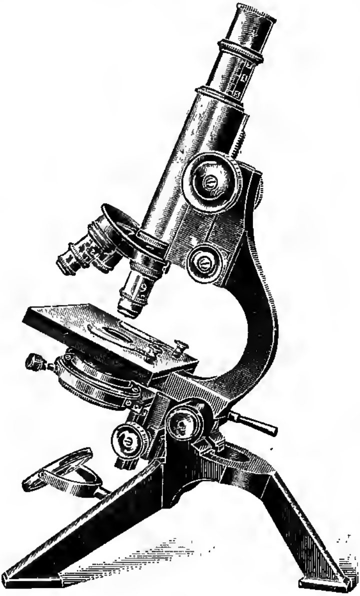


FIG. 39

Our last figure of this class of instrument (fig 41) is the H. Edinburgh student's stand of Messrs. Watson; it is here figured to show a built-in mechanical stage applied to this class of instrument. Such an addition makes a really fine instrument of any of this class, and the student cannot want a better. Any of the plain stage microscopes of this class

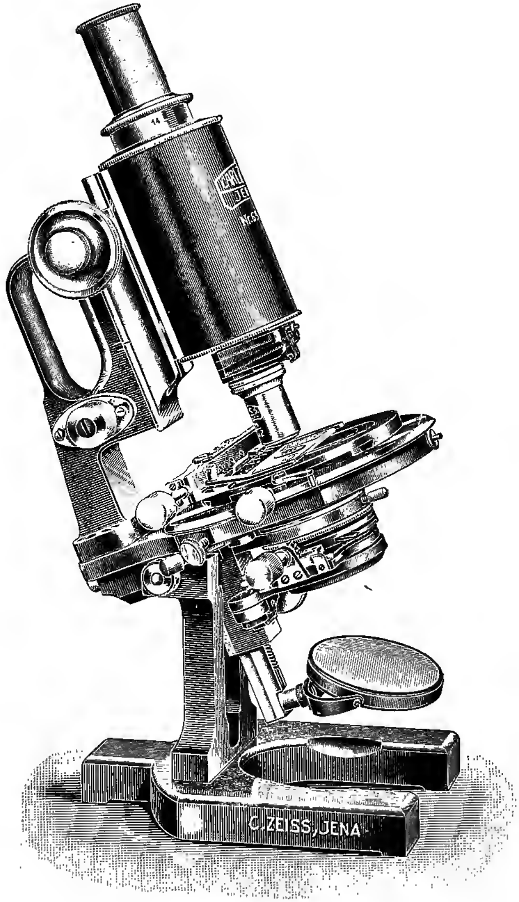


**Fig. 40.—Zeiss Large Stand with Berger Fine Adjustment**[1]

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[1] The Berger fine adjustment proved unsatisfactory and Messrs. Zeiss have discontinued making it.

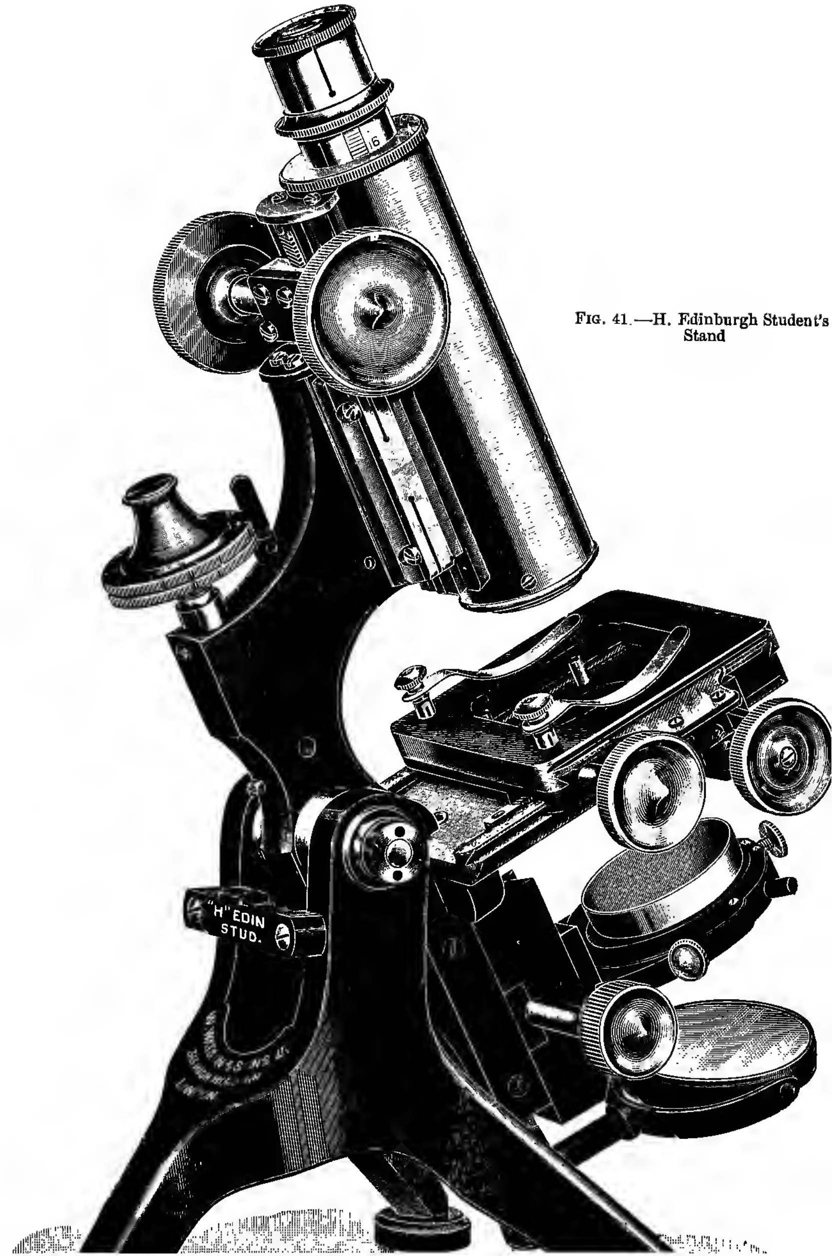

FIG. 41.—H. Edinburgh Student's Stand

may be had in case and furnished with two eyepieces, two objectives varying from 1 inch and $\frac{1}{4}$ inch to $\frac{2}{3}$ inch and $\frac{1}{6}$ inch, and an Abbe condenser for £34.

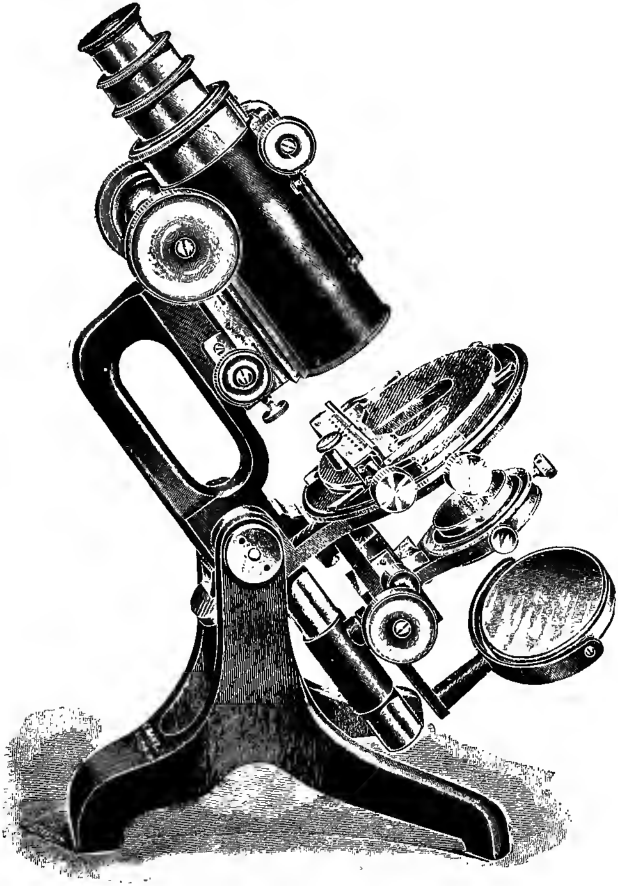

FIG. 42. —Baker's R.M.S. Microscope

Few readers of these pages will want better stands than the foregoing. Any who do so should obtain catalogues of

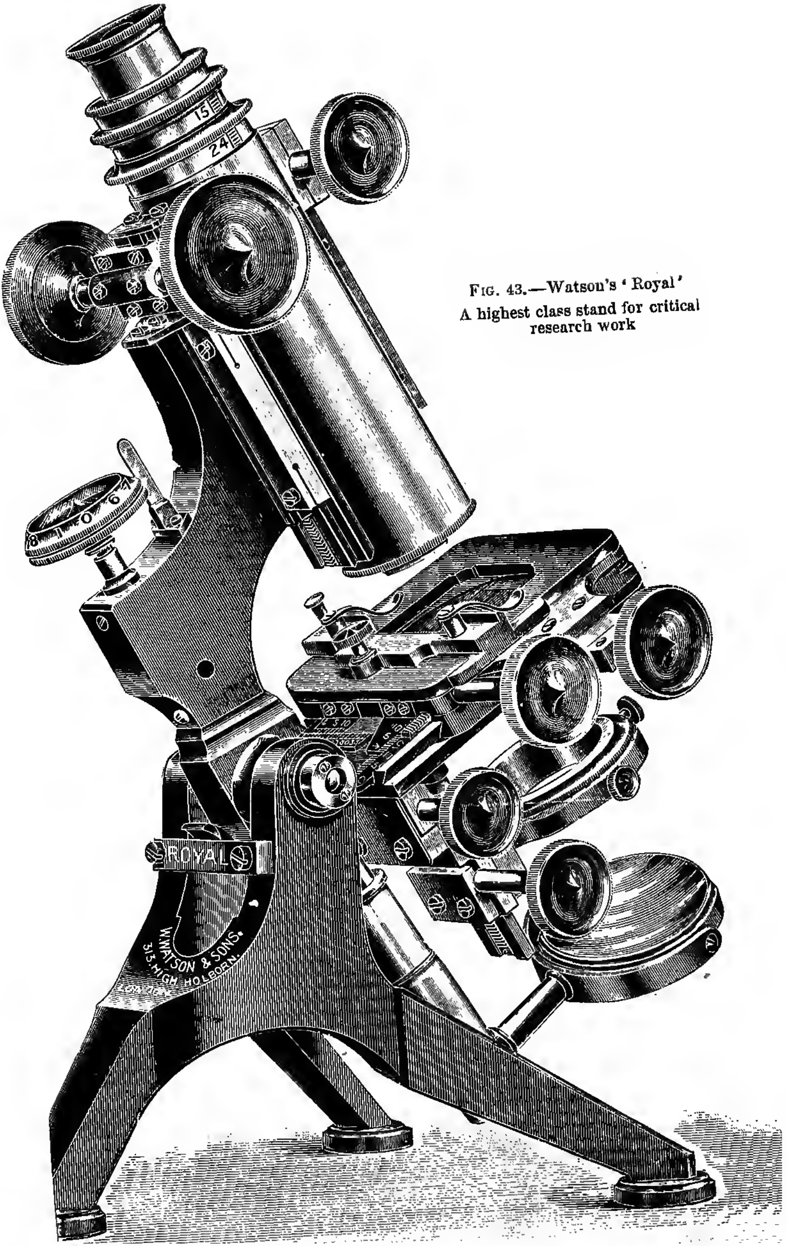

FIG. 43.—Watson's 'Royal'
A highest class stand for critical research work

the higher types of instruments designed for critical research work and made by all the leading opticians. Such stands are Messrs. Baker's R.M.S. Microscope shown in fig. 42, Swift's Universal, and Watson's Royal (fig. 43) or Van Heurck.

**Binocular.**—The best binocular instrument that we have seen is one recently introduced by Leitz and shown in fig. 44. The great advantage of this is that the microscope has a monocular body which racks completely out, leaving the objective still attached to a nosepiece. The binocular body can then be racked in, and the objective is still in focus. The workmanship of this stand is of the very highest class;

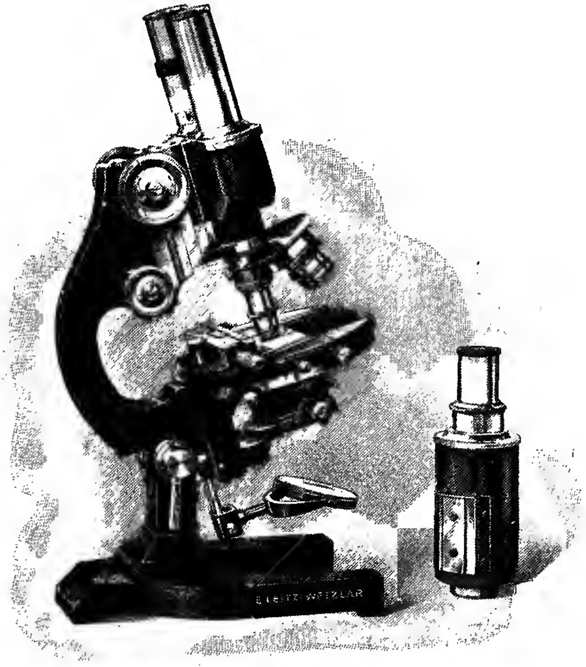

FIG. 44.—Leitz's Binocular

indeed, the whole instrument is one designed for the most critical work.

**Objectives.**—C. Baker has brought out since the war a new series of student's objectives of very great excellence. We have examined many of these and found them of wonderful uniformity. The powers and stated apertures are correct as engraved on the mounts and the working distance is good. Amongst these we may mention the 1 inch n.a. 0·20, which is an excellent lens; the $\frac{1}{4}$ inch n.a. 0·75, which we consider to be the best of its power amongst student's lenses on the market. The $\frac{1}{6}$ inch n.a. 0·84 and $\frac{1}{12}$ inch n·a. 1·3 are also excellent lenses. The prices are 38*s.* for the 1 inch, £3 10*s.* for the $\frac{1}{4}$ inch, whilst the $\frac{1}{6}$ and $\frac{1}{12}$ inch cost £3 10*s.* and £9 respectively. Messrs. Beck produce a series of student's lenses comprising a 16 mm. ($\frac{2}{3}$ inch), an 8 mm. ($\frac{1}{3}$ inch), a 4 mm. ($\frac{1}{6}$ inch) and a 2 mm. ($\frac{1}{2}$ inch). The $\frac{1}{3}$ inch n.a. 0·54 at £4 5*s.* is a fine lens and bears high eyepiecing well.

Messrs. Leitz, of Wetzlar, have always held a high position for the excellence of their objectives. The No. 3 $\frac{2}{3}$ inch n.a. 0·28 is as good of its power as it is easy to find. The $\frac{1}{6}$ inch n.a. 0·85 and $\frac{1}{12}$ inch n.a. 1·30 are also excellent lenses. Reichert's No. 7 $\frac{1}{7}$ inch n.a. 0·87 is a really beautiful lens, standing high eyepiecing extremely well and giving very crisp images: his No. 8A $\frac{1}{8}$ inch fluorite lens is also very good and not so easy to distinguish from an apochromat.

Swift & Son have always had a good name for their student's lenses: the $\frac{1}{2}$ inch n.a. 0·50 price £4 10*s.* is, we think, the best of the series and is certainly a beautiful lens; the $\frac{1}{4}$ inch n.a. 0·80 is also very good, but we think the magnifying power is really that of $\frac{1}{5}$ or $\frac{1}{6}$ inch, which is a pity.

Messrs. Watson in their series of parachromatic objectives produce some very fine student's lenses. The 1 inch n.a. 0·21 at 37*s.* 6*d.* is an excellent lens in every way. The $\frac{2}{3}$ inch n.a. 0·28 at 37*s.* 6*d.* is as fine of its power as any student's lens on the market; indeed, it comes remarkably near to the apochromats, and that is saying much. The $\frac{1}{6}$ inch n.a. 0·74 deserves special mention. This lens was specially computed by Professor Conrady to give a long working distance, and the degree to which he has succeeded may be seen by the fact that the free working distance exceeds 1 mm. The lens is a semi-apochromat, and gives superb definition and bears high eyepiecing in a manner that few student's $\frac{1}{6}$ inch will do. Moreover, the lens is true to its stated focal length; that is to say, it is a strict $\frac{1}{6}$-inch objective and not a $\frac{1}{8}$ inch marked $\frac{1}{6}$ inch. This is important, and is specially mentioned here as so many so-called student's $\frac{1}{6}$-inch lenses are really $\frac{1}{8}$ inch in power. For histology there is no doubt a true $\frac{1}{6}$ inch is infinitely better than a $\frac{1}{8}$ inch, and the $\frac{1}{6}$ will of course possess far more optical index, which is a very important point. Amongst oil immersions Messrs. Watson produce $\frac{1}{7}$ inch n.a. 0·95 which is specially computed for dark ground work, and is of great excellence, and $\frac{1}{12}$ inch n.a. 1·28 which is well known for its special merit.

Messrs. Zeiss produce a very good series of student's lenses of wonderful uniformity. The a.a. (1 inch) n.a. 0·17 is a fine lens, though of low aperture. The D ($\frac{1}{6}$ inch n.a. 0·65) is a well-known student's lens, but we consider the aperture altogether too low for such an objective. The $\frac{1}{12}$ inch n.a. 1·25, which is really $\frac{1}{14}$ inch, is in our opinion the best of the series.

The American firms, Messrs. Bausch & Lomb and the Spencer Lens Co., hold a high reputation in the United States, but their lenses are not so well known in this country.

Koristka, of Milan, has of recent years sent a number of student's lenses over, some of which are of good quality. In our opinion the $\frac{1}{15}$-inch oil immersion, which is a semi-apochromat, is the best lens of this maker.

Should the student desire better lenses than the foregoing and yet be unable to afford the apochromatics, he will find his wants met by the Holoscopic series of objectives by Messrs. Watson. These lenses are from computations of Professor Conrady and come so near to the real apochromats as to require expert judgment to discover that they are not. The n.a. is the same as the apochromats, and they bear high eyepiecing quite as well. For perfection of correction, coupled with the beautiful images they yield, this series of lenses stand alone amongst semi-apochromats.

**Apochromatic Objectives.**—These are high in price and but few readers of these pages are likely to purchase them. The Zeiss series are too well known to require much mention. The best of them are, we think, the 12 mm. n.a. 0·65 and the 3 mm. oil immersion n.a. 1·40, which are amongst the finest objectives yet produced. Whether their high price is justified is doubtful.

Messrs. Leitz, of Wetzlar, have held a high position in the manufacture of apochromats, their 2 mm. oil immersion n.a. 1·32 is certainly one of the finest lenses of this power yet made, and its price is very moderate for such a high-class piece of workmanship, being £16.

Messrs. Watson's parachromatic series of lenses have already been referred to, but since the war they have brought out an entirely new series of apochromatics which are of the very highest order. We have examined very many of these lenses and found them to be in every respect equal to the best of the Continental ones and in some respects superior. The 8 mm. 0·65 n.a. and the 4 mm. 0·85 n.a. are certainly amongst the finest dry lenses yet produced, whilst in our opinion the 2 mm. oil immersion n.a. 1·37, which has usually even more aperture than is claimed for it, is the best lens of this power on the market to-day.

One thing should be noted amongst all apochromats, and that is they are true to their designated focal lengths. A

$\frac{1}{6}$ inch is a true sixth and not a $\frac{1}{8}$ inch marked $\frac{1}{6}$ inch; in other words, their optical index is as high as possible.

**Eyepieces.**—Little need be said in this connection, the construction of the ordinary Huyghenian eyepiece has already been referred to, and this pattern is made by all opticians for the achromatic series of lenses. They vary in power from ×5 up to ×20. The most useful powers for the student to select where two are purchased will be the × 5 and × 10. The higher powers are of little use except for occasional reference purposes. The eyepiece should be marked with its magnifying power and not by arbitrary letters or figures. The apochromatic series of lenses and the holoscopic series of Messrs. Watson must be used with the special compensating eyepieces in order that a correctly balanced image be obtained.

In order that both achromatic and apochromatic objectives can be used with one series of eyepieces Messrs. Watson & Sons introduced their adjustable Holos. eyepieces which can, by varying the length, be used as ordinary Huyghenian eyepieces and also as compensating ones (fig. 45). These will be found to be superior to any of the ordinary Huyghenian eyepieces. Zeiss produces a series of compensating eyepieces numbered from 2 to 27, and these figures were supposed to represent the actual magnifying power. They were, however, only true when made for the English tube length, the same eyepieces being made of smaller diameter for the Continental tube but marked with 50 per cent. less magnifying power; thus the ocular marked × 18 for the 160 tube was really a ×27, whilst the ×12 was ×18. There is no sense in such a proceeding, and it is to be hoped it will cease.

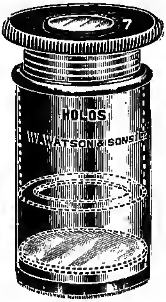

FIG. 45.—Holos. Eyepiece

## SOME ACCESSORY APPARATUS

**Illuminating Apparatus.**—It has been repeatedly insisted throughout these pages that the substage condenser is essential if good work is to be done; indeed, a microscope without such an appliance ceases to be a microscope and becomes a mere magnifying glass. Indeed, the microscope is too often regarded in this light, not only by students, but unfortunately by teachers of histology who should know better.

Fig. 46 is a section of the ordinary Abbe illuminator which is in very general use amongst medical students and their professors, though a very elementary knowledge of the optics of the microscope would soon convince them of the need for something better. It must be remembered that the Abbe condenser is totally uncorrected, and that both the spherical and chromatic aberrations are therefore enormous. Its total n.a. is 1·20 when used immersed, but the actual aplanatic cone, which alone can be used for work, is only 0·50, which although it may suffice for an objective of n.a. 0·65, seriously limits the performance of a lens with a greater aperture. For example, let us suppose a worker uses a $\frac{1}{12}$-inch oil immersion of n.a. 1·20 and an ordinary Abbe condenser; he is losing nearly 25 per cent. of the resolving power of such a lens, which is roughly the sum of the n.a. of the objective, and the aplanatic cone of the condenser, divided by 2. Thus in the present case we get $\frac{1\cdot20 + 0\cdot5}{2} = 0\cdot85$. Now suppose we use the same objective

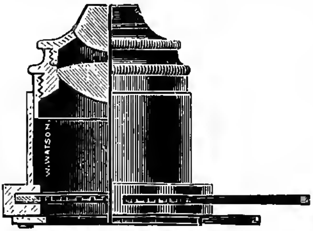

FIG. 46.—Section of the ordinary Abbe Illuminator

with an achromatic condenser giving a cone of, say, n.a. 1, we get $\frac{1\cdot20+1}{2}=1\cdot10$. The immense gain is at once apparent.

To try and improve matters Messrs. Watson brought out an aplanatised Abbe giving a cone of 0·65, which is certainly a great improvement over all the older forms. Even this, however, is inadequate for serious work, although it will meet all the student's earlier requirements with objectives not higher in power than, say, $\frac{1}{6}$ inch.

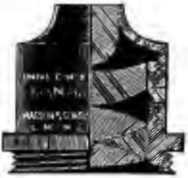

FIG. 47.—Watson's Universal Condenser

An infinitely superior condenser is the achromatic form now made by all the English firms; as an example we may take Watson's Universal shown in fig. 47. The total n.a. is 1·0; that is, the largest cone that can be passed by a dry lens. Its aplanatic cone, however, exceeds n.a. 0·95. Its corrections are most beautiful, and from long personal experience of it I can speak in the highest terms of the vastly improved images it yields. Somewhat similar condensers are made by Beck, Swift, and Baker. Such condensers will cover the whole field of the student's work. Fig. 48 shows Baker's achromatic condenser with an aplanatic cone of n.a. 0·90.

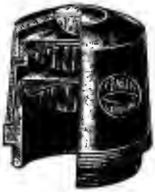

FIG. 48.—Baker's Achromatic Condenser

**Oil Immersion Condensers.**—At times when the maximum powers of resolution are required from high apertured oil immersion objectives the oil condenser becomes necessary. These vary in aperture from n.a. 1·20 to 1·40 and transmit aplanatic cones up to about n.a. 1·35. They are only necessary for the most critical work with oil immersion objectives.

**Nosepieces.**—To the busy worker these are almost essential, as they save the trouble of unscrewing one objective and replacing it by another. An object examined with, let us say, the $\frac{1}{2}$-inch lens, that requires to be examined with a higher power, simply requires the nosepiece to be rotated when the required lens is in position and only needs focussing. The most useful form is the triple dust-proof pattern shown in fig. 49. As a rule the makers fit the student's objectives so as to be approximately in focus when rotated one after the other, without any racking back of the body tube. This will not be the case when the worker uses objectives by various makers, or the higher class objectives, and must be remembered when using the nosepiece.

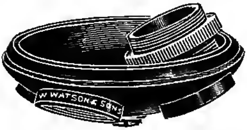

Fig. 49.—Triple Dustproof Nosepiece

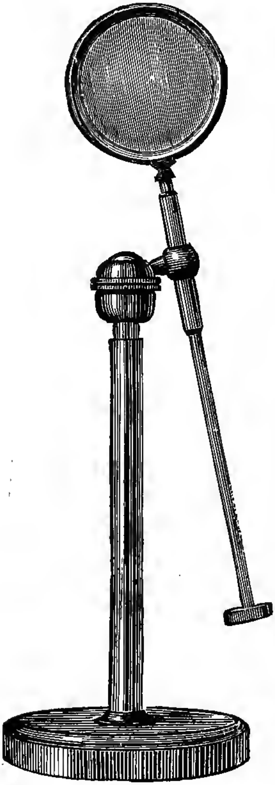

Fig. 50

The bull's-eye condenser, shown in fig. 50, will at times be useful, though it is not used now nearly so much as formerly. It may be noted that the field lens of an eyepiece makes a very useful bull's-eye condenser, especially if fitted so as to swing in front of the lamp. The bull's-eye consists of a thick plano-convex lens fitted in a suitable holder. It is chiefly useful for two purposes. One is to focus the light upon an object to be examined by reflected light. The more usual purpose is to obtain a parallel beam of light. This is done by adjusting the bull's-eye at its focal distance from the lamp flame. The flat side is turned towards the flame. It is used in this manner in working with the various dark ground illuminators. A much better form is the aplanatic bull's-eye: this is usually fitted with an iris diaphragm and yields an intense beam of light, under perfect control.

**Dark Ground Illumination.**—It should be clearly understood that beautiful as are the effects yielded by this method of examination, it does not and cannot yield anything like a critical image, and is therefore of no use for a detailed study of any object. For the detection of minute organisms such as spirochaetes or bacteria it is very useful, and it yields beautiful pictures of diatoms or polycystina. For low-power work with objectives of from 1-inch to ½-inch focus, and with numerical apertures up to 0·35, the ordinary stops sold with the substage condensers will do all that is required. Such a set of stops are shown in fig. 51. Suppose we have some polycystina to exhibit in this manner, we should proceed as follows. Arrange and focus the object in the ordinary way, taking special care that lamp, condenser, and objective are truly centred. It now only remains to put in the stop carrier of the condenser the smallest stop that gives the best effect. This must be ascertained by trial, the condenser being racked up or down a little till the best effect is obtained; if one stop does not suffice another larger should be tried. One of the best methods of obtaining dark ground with objectives up to 0·65 or 0·70 n.a. is to cut a series of trial stops on black paper till the best size is found. The stop may be fixed to a circle of glass cut to fit the stop

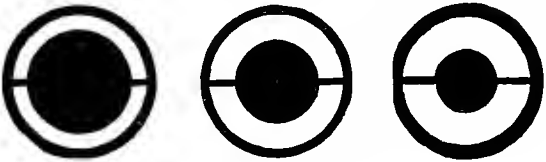

FIG. 51.—Stops for Dark Ground

carrier. With care good dark ground illumination may be obtained in this way with objectives from $\frac{1}{3}$ inch to $\frac{1}{4}$ inch. The special dark ground illuminators are constructed to work when in oil contact with the slide, and full directions accompany them as they leave the makers. A good stop with an achromatic condenser and a $\frac{1}{3}$-inch objective of n.a. 0·65 will do all that the student is likely to require. Indeed, some microscopists prefer this method to any of the special illuminators.

**Opaque Illumination.**—A great many objects are best observed in this way, *i.e.* by *reflected* light. There are various methods available.

The simplest is to turn the microscope *away* from the window or lamp, and then to focus rays upon the object obliquely by the stand-condenser; or a bull's-eye condenser is often mounted so as to attach to the stage, or limb, of the instrument itself. Often no condenser is needed, but the direct light alone from behind the observer may be sufficient. In most cases care should be taken that no light is thrown up through the slide from beneath.

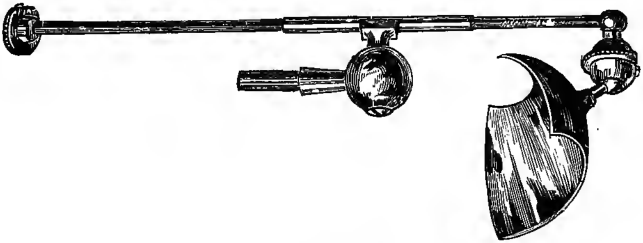

FIG. 52.—Side-reflector

The most general appliance used when more light is required is the *parabolic side-reflector* shown in fig. 52. It will be readily seen how the two ball-joints allow of adjustment: the short stem fits into a hole in the stage or limb. The lamp being placed at one side, the rays are collected by the reflector and focussed strongly upon the object, obliquely from one side.

Another method, now too much neglected, is the *Lieberkühn* reflector, fitted so as entirely to surround the objective. The action will be seen from fig. 53, where the parallel rays from beneath the stage (parallelised by the stand condenser, and reflected by the *plane* mirror) are focussed by the reflector *c* (here represented as screwed on the objective *b*) upon the object *d*. This object is here shown as held in a stage forceps *f*. No light should pass *through* the object; and this is often secured by mounting it upon an opaque black disc on the slide. These are generally much too large: the disc should stop any rays from entering the lens, but as few other rays as possible. A transparent slide may have a disc of black paper temporarily fixed at the back; or a better plan (necessary for objects held in forceps as in the figure) is to have one or two stops *e* of different sizes (often called 'dark wells') mounted in the substage.

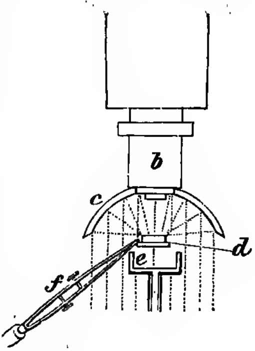

FIG. 53

By having the barrels of several lenses the same size, a Lieberkühn made as fig. 54 may be fitted to *slide* on them, and be thus 'focussed' on a slide so as to work with all. Of course this is only possible within certain limits of focus.

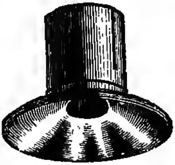

FIG. 54

**Polarised Light.**—Very few words are needed under this head; with the polariser and analyser in place, any object on the stage is at once seen 'by polarised light.' It is only necessary to say that the best effect is always with the prisms crossed, giving (apart from the object) the 'dark field.' The position on the stage will, however, affect the phenomena remarkably. It is best not to use a selenite beneath, unless it is needed; but if the effect is very faint, though visible, the selenite will often bring it out much more prominently.

# CHAPTER V

## MICROSCOPE MANIPULATION

Having got our microscope, according to our pecuniary means or general purposes, let us next consider how to use it in the proper manner so as to get the utmost from it.

**Care of the Eyes.**—This should be borne in mind, as injury might result from much work under certain conditions. The chief causes of injury are (1) either too great an amount of light, or (2) the bad habit of shutting one eye whilst using the other. Too little light might also cause eye-strain, but this rarely occurs. It is much better to use each eye in turn if possible; in any case the unused eye must be kept open. This can usually be done with a little practice, the mind not seeing, as it were, what is before the eye not in use. If there is a difficulty it can be overcome by bending a piece of wire into two rings, connected by a straight bit, as in fig. 55, the two rings being the distance of the eyes apart. One ring is covered with black paper, the other is made to fit on tightly to the eyepiece. The black screen will turn to either side for the unused eye. If

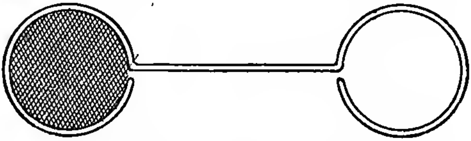

Fig. 55.—Eye-shade

the reader uses the microscope *board*, to be shortly described, such a ring will be unnecessary in any case.

**Three Great Principles.**—From the beginning let a new student accept on the authority of all microscopists of experience, and keep present in his mind, three cardinal principles.

(1) Use no greater power than required by an object. Every beginner has the tendency to magnify as much as possible, the experienced worker does the contrary. If we can see clearly all that is to be seen with a given power, we see it worse and not better by increasing the power. Of course a certain scale is necessary, and one observer can see finer detail than another; but a beginner who possesses an inch and $\frac{1}{4}$-inch powers will learn more and get on faster if he does not use the $\frac{1}{4}$ inch at all, till he has had a week or two's practice with the other.

(2) The eye needs educating. At first the student will not see half there is to be seen, with a given power, in a great many objects. If this were not so an object would only need to be seen and not studied.

(3) As much depends upon correct illumination as upon having the finest lenses. In many cases more.

**Light.**—For real work daylight is not suitable, as will become evident later, and usually the time at the disposal of the amateur will be the evening and artificial light a necessity. Probably most amateurs will find the lamp to be the most suitable form of illuminant, but it should be borne in mind that the ordinary forms of lamp are not suited to microscopical work, and the student is recommended to purchase a properly fitted microscope lamp. Fig. 56 shows one of Baker's, the price being £2.

A very good and brilliant illuminant can be made if the worker can use either gas or electricity. In the case of gas we only require a fitting that will take an inverted incandescent gas mantle. This fitting should be mounted on either a wooden or metal stand. A perforated tin (a cigarette tin will do) should be fitted so as to enclose the mantle, and this tin should be pierced with a hole about half an inch in diameter. A thin piece of finely ground glass should be fixed in front of the hole so as to get rid of the structure of the mantle. This will give a very brilliant illumination and gives no trouble.

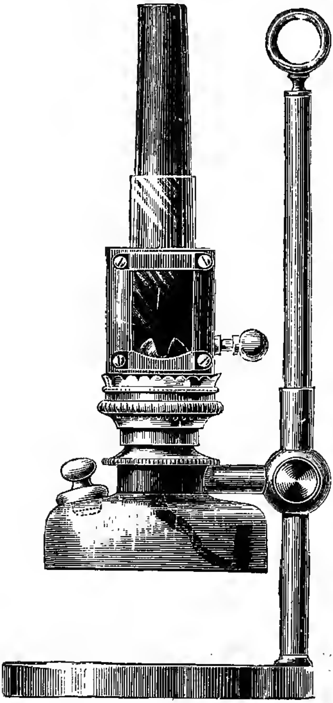

Fig. 56

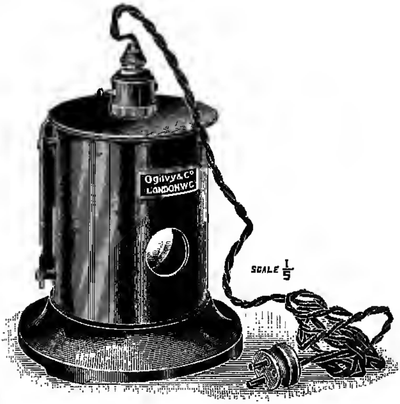

Fig. 57.—Electric Lamp

The light preferred by the writer for general work is one of the ½-watt electric lamps. This should be silvered by dipping in the following solutions. The lamp is first thoroughly cleaned by being immersed in a 20 per cent. solution of caustic soda. It is then washed in water and rinsed in dilute (1 in 3) nitric acid and again rinsed in water. Just before immersion in the silver bath it is flooded with a solution of stannous chloride, to which a little hydrochloric acid is added. The silver bath is made from two solutions, Nos. 1 and 2, which have the following composition:

No. 1

| | |
|---|---|
| Silver nitrate | 2·5 grams |
| Distilled water | 100 c.c. |

Dissolve the silver in the water and then add dilute ammonia, drop by drop, till the precipitate which is formed just dissolves. Then filter the solution.

No. 2

| | |
|---|---|
| Silver nitrate | 0·5 gram |
| Rochelle salt | 0·4 „ |
| Distilled water | 250 c.c. |

Dissolve the silver in hot distilled water, add the Rochelle salt, and boil till the precipitate turns grey, then filter.

Equal volumes of the two solutions are mixed in a large beaker which has been coated with paraffin wax and the lamp is immersed and left for a couple of hours. It is then removed and washed with distilled water and placed in a warm place to dry.

After silvering it is coated with gutta-percha, dissolved in chloroform, and a good thick coating of Indian ink applied and allowed to dry. A circular window is next made by removing the ink and silver with some cloth dipped in dilute nitric acid, and this is then frosted by rubbing it with a drop or two of Vitrograph ink (to be obtained from Messrs. Baird & Tatlock, of Hatton Garden). The lamp is then mounted in a wooden box which has a hole cut in it to exactly correspond with the illuminating portion of the lamp. This is fitted with an iris diaphragm (an old condenser mounting will come in very useful for this). We have now a most brilliant illuminant and it is under the most perfect control by means of the iris.

It is very desirable that the microscopist should have a table kept sacred to his use, though the ordinary dining-room table is frequently made use of at a pinch. A good heavy kitchen table is very convenient, where a room can be given up to the purposes of microscopic study. For the greatest comfort, and in order that fine detail shall be easily perceived, it is desirable that the room shall be in darkness, the only light reaching the eye being that through the microscope.

The best possible arrangement that gives every convenience and comfort in working, whilst at the same time allowing the room to be illuminated either by daylight or artificial light, and at the same time allowing the microscopist to work in practical darkness, is one devised by Dr. W. J. Penfold, and used in the laboratories at the Federal Serum Institute, Melbourne. This could be made by any carpenter or by the worker himself if he possesses a little skill with tools. It consists chiefly of a box-like cupboard, shut in in front by a roll top, which pulls down so that when not in use everything is covered from dust. The inside of the cupboard is provided with a stout shelf to take the microscope, lamp, and other apparatus. This shelf is at such a height that the worker can sit just within the space and use the microscope either vertically or inclined. The inside of the cupboard is painted dead black, and those in use at Melbourne have gas and electricity laid on so that any form of illumination can be used.

The comfort of such an appliance can only be properly appreciated by those who have used it, but for all delicate work and dark ground examinations it will be found to be invaluable. Where expense is not an object and the very highest type of illuminant is required the Mercury Vapour light should be used. This can be obtained from Messrs. Baker or Swift, a new pattern recently designed by Mr. J. E. Barnard being the best type. The great value of such a light is that the dominant wave length is very short, and thus greater resolution is secured.

**Microscope Manipulation.**—First let the microscopist get one or two rules firmly fixed in his mind so that they become automatic in use.

(1) *Never* use the fine adjustment where the coarse will serve. It is a bad microscopist that flies to the use of the fine adjustment for powers below the $\frac{1}{6}$ inch. One can tell the experienced manipulator at once by the way he handles his instrument. The novice uses the fine adjustment for focussing such powers as the $\frac{1}{2}$ inch, the real microscopist would never require to do so.

(2) Never use the fine adjustment by rubbing the finger to one side of it. This will soon ruin any delicate adjustment. Grasp the milled head firmly yet gently between thumb and forefinger.

Let us next proceed to set up our microscope for work. If we are using a microscope with a triple nosepiece, first screw on the objectives, arrange these in such order that the higher powers follow the lower ones thus, 1 inch, $\frac{1}{2}$, $\frac{1}{4}$; this is convenient for rapid work and saves much time. Next place a low-power ocular ×5 in the tube and the substage condenser in its sleeve. We are now ready to attack our object. Let us suppose this to be the proboscis of the blow-fly. I will assume first of all that the worker is using the oil lamp. This must be lit and arranged on the table so that the edge of the flame is facing the microscope mirror.

Place the microscope in front of the lamp and some 10 inches distant. Next see that the mirror and tail rod is central and incline the microscope to a comfortable angle, say about 45°. Place the slide centrally on the stage and rotate the 1-inch lens into position. Looking down the ocular, incline the mirror (which should be the plane one, when the condenser is used) till the light of the lamp is seen illuminating the field. Now gently lower the inch objective by means of the coarse adjustment, looking down the tube whilst so doing till the object comes into view. Some beginners find difficulty in doing this. In such cases arrange the object so that it appears to be central with the front lens of the objective, then rack the objective down till nearly touching the slide. Having done this rack slowly upward, watching through the ocular. Some part of the object will probably come into view, or if out of centre dust will be seen on the cover glass when the object may be moved into the field. Having found the object, accurately focus it by means of the coarse adjustment. At this stage try and forget the microscope possesses a fine adjustment at all.

Having focussed the object, we must next attend to the illumination. If the microscope be provided with a proper rackwork substage, we must rack the condenser slowly up till the image of the edge of the lamp-flame comes clearly into view, with that of the object focussed on the stage.

It will next be necessary to centre this correctly. The easiest way of doing this is to first close the iris diaphragm to as small an aperture as possible and then to rack up the objective till the aerial image of the iris is seen; of course it is necessary to look through the ocular while doing this. Having found the image of the iris, centre this accurately by means of the centring screws of the substage.

Focus the object with the objective again. It will probably now be noticed that the image of the flame is not central. Move either the lamp or microscope till the flame image cuts the field into two equal halves or is exactly central.

In microscopes with only an understage fitting the same method is to be pursued, except that the condenser will have to be moved up and down to focus by hand, and exact centring is not possible. In such cases it will probably be found that if the condenser be rotated it will be central at one part or other of the rotation. If the lenses are purchased from the maker of the microscope, the condenser is usually centred approximately to them. There is, however, no means of doing this really accurately except with a proper compound substage.

Let us next examine the object with the ½-inch power. Rotate this into centre with the object. (It must be first ascertained that this can be rotated into position without racking up.) Focus the object again as described for the inch power. It will probably be observed that the image of the lamp-flame is no longer central. If this is so, move the centring screws till it is once more central, but do not on any account move either mirror or lamp. Note particularly that having once centred the iris and put the condenser into centre with the low-power lens by moving mirror, lamp, or microscope, any change of centre occasioned by the use of other lenses must be corrected by moving the centring screws only. The lamp or mirror must not be touched, or we shall get oblique light and not central. Different objectives have different optical centres, and so each objective requires the condenser to be centred to it.

We have now focussed our object—also focussed and centred the condenser. Are we yet in a position to start the examination of our object? Decidedly not. We must first arrange the quantity of light to be used, and next we must see that our objective is exactly adjusted to the object.

With regard to the amount of light or cone, as it is termed. Remove the eyepiece and examine the back lens of the objective. It will probably be completely filled with light. Our object is to close the iris till about three-quarters of the back lens are filled; this is termed Mr. Nelson's three-quarter cone, and most lenses perform at their best when so adjusted. If on replacing the eyepiece and examining the object we find the flood of light too great, we must reduce it by closure of the iris; but the more nearly we can use the three-quarter cone the better the image will be, provided that the object will bear it. Many objects such as living Infusoria will not, and in such cases we must reduce the cone of light till good definition is obtained.

For objectives lower in power than the half-inch the condenser is not necessary; plenty of light can be obtained by using the flat of the flame and the concave mirror. If the condenser be used for such objectives the top lens had best be removed. In many cases the light can be subdued by placing (either in front of the lamp or in the stop holder of the condenser mount) a piece of blue glass; this usually tones down the light to an agreeable tint. Where either gas or electricity is used in place of the lamp the same procedure should be followed, but the image of the diaphragm before the illuminant should be focussed by the condenser.

The partially illuminated field often seems strange to the beginner, but he should bear in mind that our object is to secure the finest definition and illumination in the centre of the field and the rest is neglected. The whole field may be usually filled with light if desired, either by using the bull's-eye to magnify the source of light or at times by turning the flat of the flame to the mirror. In cases where the gas or electric lamp is used we can open the iris of the light. If the worker is using the Abbe condenser, so common amongst students, the total aplanatic cone of light he will obtain will only be n.a. 0·5. This, however, will be sufficient for most student's objectives up to about n.a. 0·70.

Let us examine the method of ascertaining this aplanatic cone. Place either the blow-fly's proboscis or a slide of *Pleurosigma angulatum* on the stage and focus both objective (say the $\frac{1}{6}$th of n.a. 0·65 to n.a. 0·74) and condenser as already described. Push aside the object and remove the ocular and look down the microscope tube. The iris aperture shows as a bright ring of light in the centre of the back lens (fig. 58). Open the iris and the bright circle will increase (fig. 59). This circle, when increased as much as will remain unbroken, will give us the aplanatic cone of the condenser, and in the case of a $\frac{1}{6}$-inch objective having an n.a. of 0·65 would with the Abbe condenser give about a three-quarter cone. If the condenser be racked up further

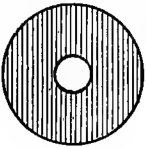

FIG. 58

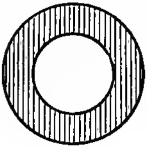

FIG. 59

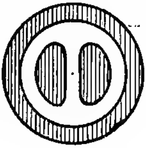

FIG. 60

this cone of light breaks as in fig. 60, and only the largest unbroken cone of light can be used for critical work.

As made at present very few if any lenses will stand a full cone of light (*i.e.* a cone equal to their own aperture) on most objects, many will not stand a three-quarter cone. The better the objective and the condenser the more cone can be borne with advantage, always provided that the object itself will bear it. With very transparent objects the cone may have to be cut down more and more simply in order that the details may give sharper shadows. By the smaller cone we get the necessary light and shade, but at the same time we thicken and blur the image. Thus the microscopist has to employ different expedients and sometimes to put up with one imperfection in order to fight another, and so on. It will suffice for most present readers to make a careful study of the cone of light at the back of their objectives when the eyepiece is removed, and to aim at the best images with the largest cones that they can obtain.

**Adjustment of the Objective.**—Let us next consider the question of getting our objective into correct adjustment. We must remember that all objectives are made to work, firstly, at some specified tube length, and, secondly, with a cover glass of a certain definite thickness. If an objective were made to work, or corrected, as it is termed, for an uncovered object, it would become over-corrected when employed with a thin cover glass, and the thicker the cover glass the more over-correction would be apparent. The reverse, of course, holds good. An objective corrected for a definite thickness of cover would become under-corrected if it were employed with a thinner cover. We can cause an objective to become over-corrected by lengthening the draw tube of the microscope and under-corrected by shortening it. We have therefore the means of adjustment at hand.

How shall we know whether our lens requires any further adjustment? Let us suppose we have focussed both objective and condenser as already explained. Move the slide about slightly, looking down the microscope tube. We shall probably find a small speck of black somewhere about, or if not, some dark portion of the object which is strongly marked. Having found this, alter the focus up and down. If the lens is not in proper adjustment it will be found that the speck will open out into a darkish ring when the focus is shifted down, and will fade into a misty fog when the focus is shifted above. Should this occur it indicates that either the cover glass is too thin or the tube length not long enough; in other words it indicates under-correction. Where over-correction is present or a too thick cover glass or too long a tube length, we should find the ring appearance when we focussed upwards and the misty when downwards.

How, then, shall we set to work to perform our adjustments scientifically? Let us suppose the cover glass is too thin. First get the rings round the speck chosen, then, without touching the coarse or fine adjustment, slowly lengthen the draw tube till the exact focus of the object is restored. Test again to see whether rings still appear when focussing downwards. If they do, repeat the lengthening till we get similar rings both above and below the focus. Our objective will now be in correct adjustment. Where the cover glass is too thick reverse the procedure.

A convenient rule by which to remember the correct adjustments is—ring down, lengthen tube; ring up, shorten tube.

Lenses lower in power than the $\frac{1}{4}$ will probably not be very sensitive to variations of cover-glass thickness unless they are really high-class lenses with large apertures. The $\frac{1}{4}$ or $\frac{1}{6}$ inch is the best for the student to practise correction with. Where a lens is provided with a correction collar exactly the same method should be employed, except that the lens should be first of all set at the tube length for which it is corrected, and the alterations for restoring the focus be performed by means of the collar and not the draw-tube.

We may now study our object under what is termed critical illumination, and provided our lens is a good one we shall have a critical image. This term was invented by Mr. Nelson to describe a sharp, perfect image of minute structure. Every microscopist should try as soon as possible to educate himself to distinguish between a critical image, only possible with good lenses and large cones of illumination, and the coarser images from inferior lenses and narrow cones.

# CHAPTER VI

## MICROSCOPICAL DRAWING, MEASUREMENT, AND PHOTOGRAPHY

**Drawing.**—This is performed by some modification or other of the *camera lucida.* The simplest, cheapest, and easiest for a beginner to use is that known as Dr. Beale's neutral-tint reflector (fig. 61). The usual cap of the eyepiece is removed, and replaced by that in the figure, outside which is a simple mount which supports a piece of transparent neutral-tinted glass at an angle of 45°. The microscope is adjusted with its tube horizontal, and, if the stand be small, should be supported on a book or box, or the stand suggested on page 106, so that its centre is ten inches from the table, on which the paper is laid. The image is then *reflected* upwards to the eye looking straight downwards, and which also perceives the paper and pencil *through* the tinted glass. This apparatus costs from 5*s.* to 7*s.* 6*d.*, the latter sum procuring several glasses of different shades.

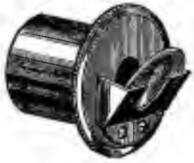

FIG. 61.

Various more costly prism arrangements are also in use, and Abbe's camera lucida has a reflector which enables the microscope to be used at any angle. Such arrangements will be found figured in first-class catalogues, and need not be particularly described here. Some are rather difficult to use; but it is singular that one observer will find most easy, the very one which another finds most difficult. Only one practical remark need be made, viz. that success depends much upon the *relative brightness* of the image as seen on the paper, and of the paper and pencil themselves. Hence a separate lamp to illuminate the paper is often desirable, and upon increasing or diminishing the light of either, comfort and success may often depend.

**Measurement of Size.**—The real sizes of minute objects are easiest measured by the aid of a *stage micrometer*, costing about 5*s.* This consists of a line ruled on a glass slide, and divided by cross lines into small spaces, usually the $\frac{1}{100}$ and $\frac{1}{1000}$ of an inch, or $\frac{1}{10}$ and $\frac{1}{100}$ of a millimetre. The object being conveniently adjusted, its leading dimensions are sketched on the paper (a mark or two usually sufficing). Then it is replaced by the micrometer; and the image of the small spaces being made to coincide, gives at once the dimensions of the details of the object.

Another method is to employ an *eye-piece micrometer*, containing a glass scale in the field of the eye-lens, and which is consequently seen in the same plane as the image.

The stage micrometer is then imaged (of course this should be done with the same power and tube-length employed on the object) and the value discovered of say one or more $\frac{1}{100}$ths of an inch, on the larger divisions of the eye-piece micrometer. Then the dimensions of the object are known by comparison. Cheap eye-piece micrometers on circles of glass are sold (fig. 62), which can be dropped into ordinary Huygenian eye-pieces. An eye-piece with micrometer fitted is much more expensive.

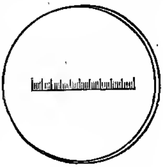

FIG. 62.

**Measurement of Magnifying Power.**—This also is simply effected with the aid of a stage micrometer and the camera lucida. The paper must be placed at the proper distance of 10 inches (p. 12) from the neutral-tint glass. Then the image of the micrometer is focussed and dimensions sketched on the paper. The most convenient division so sketched, has then simply to be measured to give the magnification. Thus, let us suppose the division known to be $\frac{1}{100}$th of an inch measures when drawn $3\frac{1}{2}$ inches, it is obvious that the magnification is exactly 350 diameters.

**Photography.**—The highest class of photo-micrography demands costly apparatus, the very finest lenses, and the highest manipulative skill, and to enter into it is out of the question in these pages; we can only give a very few hints towards success in earlier and simpler attempts, which may, perhaps, lead on towards more ambitious work. In that case, elaborate treatises on this special subject are accessible to the student. The delicacy of such work as highly magnified photographs of the more difficult *bacilli* may be gathered from the fact, that it is hopeless to attempt it except on the solid ground, in situations practically free from the vibration always present in the crowded parts of large cities.

Micrography having additional difficulties of its own, the operator should first of all have sufficient ordinary camera practice to master the ordinary *photographic* details. He should learn to judge by the behaviour of his plates whether the exposure has been correct, too long, or too short; and how to 'humour' development according to these circumstances. It is also advisable that he should become familiar with the use of the quicker plates, and especially of some of the best brands of *isochromatic* plates, which latter give far better results with many insect subjects, or with others of difficult colours.

The microscope for early work need not be expensive; but must be steady, capable of being balanced with the tube in a horizontal position, and should have a focussing and centering substage. Some amount of skill in proper illumination and display of the objects to be photographed must also be acquired; for if the visual image be not good, it is impossible for the photograph to be so. These points are almost self-obvious; but observation proves that dozens of plates and much time are absolutely wasted in efforts that *cannot* yield good results, for want of attending to them.

When thus ready for actual microscope work, there are two general methods of operation; but in either case, with most magnifications it will be needful to provide a thick and solid base-board four to six feet long, which is all the better if provided with strips on the edges of the top surface, to form a trough or slide for the apparatus to move in, easily but in line. Along one edge of this, in two or more eyes, should revolve easily a brass rod or tube, with a pulley fixed on it, round which and the milled head of the fine adjustment passes a cord or tape. Usually it will be a tape; but when a microscope is intended for photography, a groove is often turned in the milled head, and a cord used in the groove. The purpose of this is, that when the image which has to be focussed is too far off from the microscope for the operator to reach the fine adjustment with his hand, it may be worked by turning the rod, which has a milled head on the end. A very solid table should be used, on the most solid floor that can be found; but Dr. Charters-White says that on such a table he has found that vibration is largely prevented by supporting the base-board on two piles of unbound magazines.

The first method of working was recommended some years ago by the gentleman just quoted, and consists essentially in placing the microscope and lamp themselves in a box open only at one side, which also can be closed *entirely* by an efficient black curtain; and with an aperture in one end opposite the end of the microscope body. Over the lamp must of course be also a chimney, which must be light-tight during exposure. The aperture through which the microscope protrudes is easily made light-tight by fixing outside a short tube, round which and the microscope tube a loose tube of black velvet or cloth is kept tight by india-rubber bands. Outside the box is provided any kind of moveable stand to carry the focussing screen and plates, which can be adjusted at any distance from the microscope required, according to the size of image.

Large objects with low powers may be focussed on an ordinary ground glass; but for minute detail this will not be sufficient. In some cases it answers well to have white paper pasted on the front side of a glass plate, and look *at* the image, projected as on a white screen. One advantage of this method is that no focussing rod is needed, as the fine adjustment can usually be reached, while getting a critical view of the image on the white paper. But the usual plan is to use a glass plate, on the face of which (corresponding to the film in position) are ruled some fine lines, and to carefully focus these and the image together with a 'focussing-glass.'

The procedure with this sort of apparatus is pretty obvious. The lamp, substage condenser, and microscope are carefully centred, and the illumination carefully adjusted in the usual way, till the very best image possible is attained, and the screen is so adjusted as to get the image the required size and sharply focussed. Often it will be best to project it direct from the objective alone, at several feet distant. In other cases an eyepiece may be employed, at a shorter distance. The best results of all, for high powers, are obtained with 'projection' eyepieces, but these are unsuited for low powers, unless apochromatic.

Thus far the side of the box is left open to give light in the room: now the curtain must be closed and *the whole left for ten or fifteen minutes.* All will then probably appear out of focus, owing to the expansion of the metal by the heat inside the box. This must be readjusted: and not until the image appears to remain stable should exposure be attempted. Then the plate is placed in position, and a loose cap over the microscope end; the shutter removed (all being now in total darkness), and finally the loose cap removed and the exposure given, by watch. Correct exposures must be learnt by experience; but a pretty good average exposure to start trials with, at say 100 diameters, with ordinary lantern plates, may be 20 to 30 seconds.

Many good ordinary objectives may be made to give fine definition, equal to that of most apochromatics, by interposing a sheet of Chance's 'signal-green' coloured glass. The time of exposure is, however, increased considerably. Other photographers have obtained splendid results from various coloured screens and *isochromatic* plates. These last give the best results with the more difficult insect subjects.

With low powers, the ground-glass method of illumination will often be found of great use. With high powers it absorbs too much light, and reliance must be placed on carefully-adjusted central cones, as large as the lenses and the object will bear. In all cases the photographer should be careful to stand or sit still during exposure, as walking about the room would probably spoil the image by the vibration so caused.

The other method of work is to place the lamp and microscope free on the base-board, and an ordinary camera with its dark slides at the other end, removing its lens, and making a light-tight connection between its tube and microscope, in the way already described. With the usual short cameras, an eyepiece will be necessary to get a sufficient magnification; but additional bellows, up to several feet in length, can be obtained for a small sum. To use these the focussing-rod will of course be necessary. Fig. 63 shows such an arrangement in conjunction with the simple stand illustrated in fig. 31, with an ordinary lamp, and bull's-eye to supply parallel rays. Mr. E. M. Nelson has devised and described a simple arrangement of light-tight boxes open at both ends, blackened inside, and fitting into one another, with blackened internal flanges. With these a camera of any length can be built up, according to the number of boxes used end to end, which could be made by any amateur carpenter.

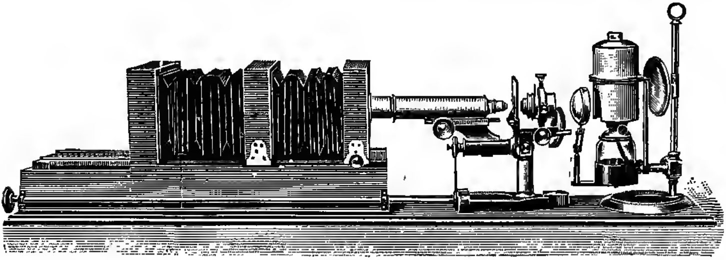

FIG. 63.—Microscope and Camera

This latter method of work (i.e. with a camera) is generally preferred by practical men. It allows the room itself to be well lighted throughout, and gives facility for using a lime-light jet, or any other powerful method of illumination.

And the apparatus becomes far less heated than when itself enclosed in a dark box, though it will be found that even the proximity of the lamp will affect the focussing somewhat, until the whole has been some time in position.

The majority of good modern objectives are fairly well corrected for photography. Nearly all are corrected and sharp in focus, if a signal-green glass is used.

In photographic work, it is of the utmost importance to prevent all 'flare' from bright *reflected light.* Some otherwise good objectives reflect a great deal of light from bright brass round the edges of their lenses; all lens mounts should be blackened. The larger in diameter the tube of the microscope is, the better; and this should be carefully blackened inside with black velvet; no mere varnish is 'dead' enough to prevent all reflection from the sides of the tube. Or another plan as good or better, is to cut several discs of card of a size which will jam or stick anywhere in the tube; cut apertures in the centre, of rather different sizes, the largest slightly smaller than the field-lens of the eyepiece; and make them dead-black with suitable varnish or Indian ink. The smallest is pushed two-thirds down the tube, and two others spaced so that the last and largest is about an inch from the bottom of the eyepiece. These side-stops catch all the surplus side-rays. The bellows or camera-boxes should be protected in the same way, square-shaped stops being easily placed at intervals along the camera.

Many plates produced are the veriest rubbish (unless of some rare or unique object, of which *any* photograph may be better than nothing). It should be understood that in this branch of photography there is as much room for taste and manipulative skill as any other. Of some objects the sharpest definition and the sharpest contrast in black and white may be desirable; but in others good half-tones, harmony of shades, and general *effect* are as important as in other subjects. Exquisite slides can be produced of suitable objects by the 'dark-ground method' of illumination. Then the *thickness* of the microscopic object has to be considered. If very appreciable, only certain portions can be 'focussed' at one time by a high-power objective; and if tolerably high magnification is required, it must be obtained by using a power low enough to get 'depth of focus,' and making up the magnification by either an eyepiece, or greater distance of the plate.

These difficulties and refinements should not be encountered all at once, but the first attempts made upon easy and flat objects, of which a flat image can be at once obtained. Sections of the stems of plants, and the wings of some insects, are very good objects to commence with. If serious and systematic work is then determined upon, the student will do well to obtain one of the detailed manuals concerning this branch of microscopy.

# CHAPTER VII

## MANIPULATION, PREPARATION, MOUNTING, AND SELECTION OF OBJECTS

Whole treatises have been written upon this division alone: we must be content with those elements suitable for a beginner.

**Natural Objects.**—Very many objects are well worth examination in their simple, natural state. Small flowers whole; their parts; insects and parts of these—all such will well repay observation as found. For this purpose an indispensable appliance is the *stage forceps*, fig. 64. The stem of the forceps fits into a hole in one corner of the stage, or the limb of the microscope, as most convenient;

and the slide, combined with the universal motion, enables a small object to be placed in any position, angle, or direction desired.

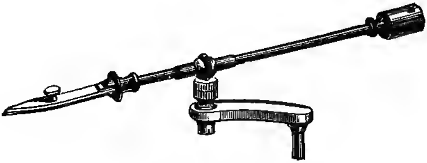

FIG. 64.—Stage Forceps

Most objects examined in this way are illuminated as opaque objects, by the bull's-eye alone; but the parabolic reflector (p. 100) or the Lieberkühn can also be used. Some may be transparent, such as the wings of many insects; and for these the concave mirror or condenser may be used as usual.

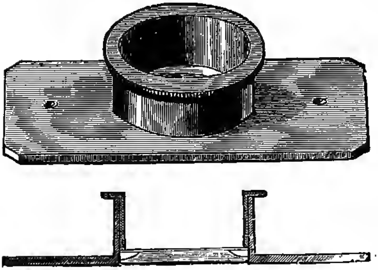

FIG. 65.—Live Box

**Live Objects.**—One way of examining live insects has already been described on page 43, but only serves for the head or mouth organs. Another method is more generally useful. A flea or other insect may be stupefied by the vapour of alcohol, ether, or chloroform, and while unconscious attached by a particle of gum on its side to a very small disc of paper gummed on the centre of a glass slip. When it recovers, the movements of any part can be well seen, by aid of the parabolic reflector or a Lieberkühn.

Another very usual method is to employ the simple apparatus called the *live box* (fig. 65). On a slip of brass is fixed a short wide tube, in one end of which is mounted a circular plate of glass. Another short tube sliding a little stiffly, either in or outside the other, has mounted in one end a thinner plate of glass; thus with care the two glasses can be adjusted at any distance desired, so as slightly to compress an insect or small animal between them. This apparatus is made either with the glasses at the bottom of the tube, or at the top, for convenience, and costs 5*s.* to 8*s.* Mr. Rousselet's form is best, in which the glasses are at the bottom of a much wider cell, so that an objective can focus anything up to the very margin of the glasses. More perfect contrivances are made at higher prices, with which the pressure can be more precisely adjusted.

**Pond and Marine Life.**—Living objects in water are handled in various ways. Infusoria, and many Rotifers, are best examined by simply putting a drop of the water containing them on a glass slip, and a loose cover-glass over, which is held on by capillary attraction. If so large as to be crushed by this, a filament of wet cotton, or of some confervoid growth, will keep off the pressure, and yet confine the movements of the animals; or it is very usual to employ the live box, enclosing now a drop of the water between the two glasses.

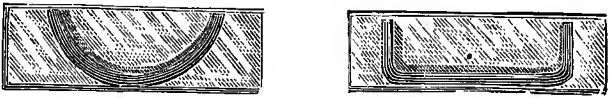

FIG. 66.—Troughs

But the majority of creatures will require *glass troughs,* the smaller of which, resembling fig. 66, can be obtained from 1*s.* each upwards, and consist of cells cemented on ordinary 3 × 1 glass slips. Thinner cells, and with thinner front glasses than can be generally purchased, will, however, often be required; and I have been in the habit of making mine. Provide half an ounce of cover-glass, about $1\frac{1}{4} \times \frac{3}{4}$ inch, for the fronts of the troughs. For the thickness, or wall, half of an ordinary vulcanised-rubber ring of suitable size will do; or for very thin troughs, a wall cut out of thin card. Either may be cemented to the slip and the front quite securely, by the ordinary balsam and benzol.

Another good way is to use glass slips with concave cells ground out of the centre; these provide a certain depth of liquid, over which a cover-glass can be placed. Such cells are sold in great variety, and also with subsidiary cells and grooves, so arranged at their sides that a current of aerated water may be maintained if necessary. These devices are known as 'life-slides,' and after a little experience the manner of use will suggest itself. A great variety are figured in Beck's larger catalogue.

For larger animals, Polyzoa, &c., large troughs are sold, of all sorts of shapes and sizes. When a trough is rather deep, and an animal has to be kept near the front, this is done by wedging up a slip of glass behind it. Marine life attached to weeds requires rather a large trough. What is called 'Botterill's' trough consists of two glass slips, with half a vulcanised-rubber ring pinched between them, or two layers if required, by means of two outer plates of vulcanite and set screws. Personally, I have found made troughs most convenient.

Vegetation, with or without life attached, is best arranged in a trough with a slender pair of forceps or needles. Separate animals may be withdrawn from the water they are kept in by a 'dropping tube.' The tip of the forefinger is held tightly over the top end of an empty tube of suitable size, and the lower end being brought over the animal, when the finger is lifted the water rushes up and carries the creature with it. Replacing the finger, the whole is lifted out, and removing the finger again will deliver the animal in the trough or on a slide. But by far the most convenient implements are the 'fillers' sold with stylograph or fountain pens; if a few are at hand with different bores at the point, small animals can be captured and again expelled with the greatest facility.

**Dissecting.**—The minute details of many objects have to be carefully separated in order to be examined. The tools needed for these manipulations consist of fine pointed scissors, forceps (fig. 67), needles for teasing-out fine structures (fig. 67), and dissecting knives (fig. 67). Forceps are required both straight and curved, and are usually of metal; but for wet work I prefer them with ivory tips. Fig. 67 shows three ways of mounting needles in mechanical handles. But it does just as well to provide some cedar handles, as used for camel-hair pencils, to bind one end round with thread, and having made a hole in the end with the point of a needle, to push the butt-end firmly in with a pair of pliers.

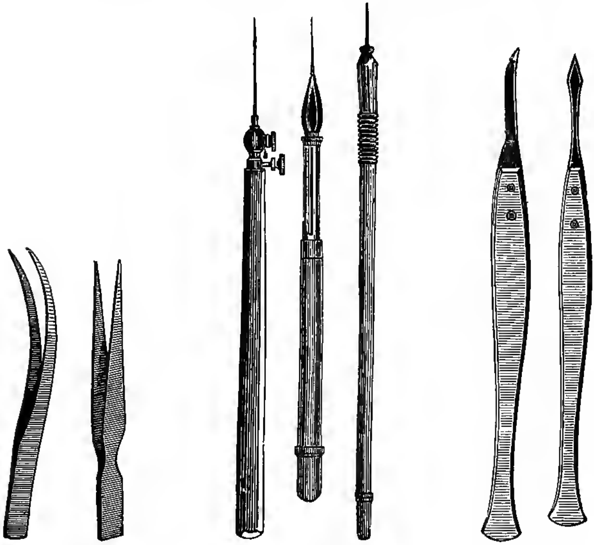

FIG. 67.—Forceps Needles Knives

There should be several straight needles of various thickness, one or two curved, and one or two bent quite to a right angle and into hooks. Knives need no description. A few camel-hair pencils, with perhaps one or two sables for their greater stiffness, are also required.

In dissecting under the simple microscope, the hands will require some support, which can be obtained from blocks or books at the sides; but the instrument shown on page 41 is provided with hand-rests, as are those of other makers. A portion of vegetable tissue, previously macerated in water, may be simply placed in a few drops of water upon the glass stage. When more fluid is required, watch-glasses may be used, cemented for steadiness into apertures cut in a plate of cork. Or for 1*s.* apiece can be obtained thick plates of glass with deep concave basins ground out and polished, which are very convenient. For some purposes it is needful to use square flat-bottomed glass troughs. It is not always necessary to work under the microscope; with light thrown on the object from the bull's-eye condenser, a great deal can often be done by the naked eye, or with rather short-focus spectacles (fig. 68).

Many objects, such as an insect, have to be fastened down to be dissected. A plate of cork loaded with lead is often convenient. But it is sometimes better to run into the bottom of the trough some waxy material, into which pins may be thrust. A mixture of paraffin and stearine will be tolerably transparent. Where transparency is not needed, beeswax softened with tallow may be used, and if desired can be easily coloured dark with lampblack. Sometimes an object is best fixed by melting a portion of the ground material with a hot wire, and slightly embedding the object in the melted surface; in other cases, or on cork, pins must be used.

Generally the liquid in the trough will be water. Sometimes diluted alcohol is better, especially in dissecting the nervous system of insects, as the alcohol hardens the tissues. Some tissues dissect best in glycerine and water.

Subjects from the plant-world have to be prepared for dissection by long soaking in water to soften them; often that is not enough, and solution of caustic soda of greater or less strength is employed; but this acts very powerfully, and the progress must be carefully watched. Insects to be dissected should always be kept in fluid till wanted; diluted methylated spirit being the most generally suitable.

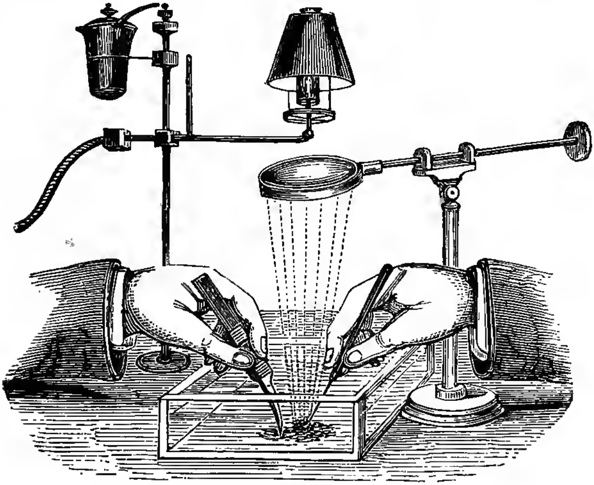

Fig. 68

**Mounting Slides.**—It will best serve the purposes of this work to select from the countless methods of mounting microscopic objects, three only, most suitable for the beginner, and most widely applicable.

Microscopic objects are almost universally mounted now upon glass slips 3 × 1 inches. Wooden slips warp, and are discarded even for opaque objects. The paper covers once used have also quite gone out, and the microscopist should employ only good flatted glass slips with ground edges. Over the objects are placed thin 'cover-glass,' which can be had either in largish oblong pieces for large objects, or in circles and squares of various sizes.

Before use, all glasses must be cleaned. For very high-class and minute work, strong solutions are often employed to do this; but for the class of objects here in view, the glass will be sufficiently cleaned with a little soap or soda, rinsing in clean water, and drying with glass-cloth, finally polishing with clean linen or a chamois leather. To polish the cover-glasses, after a little experience the finger and thumb may be used with the linen or leather, moving the cover by its edges between them, with finger and thumb of the other hand. Many use two flat pieces of wood with a chamois leather strained over the face of each; the cover-glass is placed on one, the other applied, and the two twisted together. The cover should be reversed, in each case, as it usually sticks to one of the leathers, and is only rubbed by the other.

**Cement and Cells.**—A very great variety of cements and varnishes, and materials for cell-making, are used by various microscopists; but for the processes here described a few will suffice. In regard to the walls of cells, very shallow or thin cells are built up with varnish alone. For this process, and for sealing in some cells, some kind of *turn-table* (fig. 69) is absolutely necessary. That shown is the simplest, and is known as Shadbolt's, the glass slip being simply held down by two strong springs, *a*, *b*, while the table with the slip on it is turned round by the finger on the milled ring, *c*. Other forms are chiefly distinguished from this by

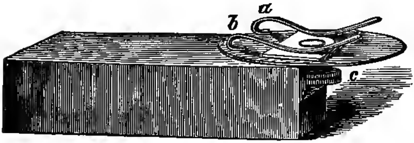

FIG. 69.—Cell-making Instrument (Shadbolt)

some arrangement or other for automatically centering the slip, so that it shall turn round the centre, and for holding it more tightly. There is, however, no difficulty in centering even with the Shadbolt form. It will be understood in an instant how, if the slip be turned round with the left forefinger, while a camel-hair or sable pencil with some varnish be held touching the slide, a circular ring of the varnish will be laid on. For very minute objects, such as the scales of some insects, one thin ring of varnish lifts the cover-glass sufficiently high ; if more be required, this coat is allowed to dry, and a second laid on the top of the first ; and even a third or more.

Deeper cells require a solid wall of some kind. Rings of glass, tin, celluloid, and other materials are sold for the purpose. Rings may also be used stamped out with a punch from paper or card, and soaked in melted solid paraffin. Without this precaution damp would enter the cell if dry ; or fluid contents might evaporate.

Of cements and varnishes it will suffice to commence with a bottle of 'gold-size,' another of asphalt and caoutchouc in naphtha, called 'black varnish,' and another of somewhat similar varnish known as 'black Japan,' or, what is now preferred by many mounters, the 'Club Black Enamel,' sold for bicycles by the Silico-Enamel Company.

**Mounting 'Dry' Objects.**—By 'dry' preparations are meant such as are only protected from dust by a cover-glass, and not immersed in any fluid medium. Nearly all opaque objects are so mounted, and many transparent ones may be treated the same way, such as scales from insect wings, diatoms, leaves, and petals of plants.

Many of the latter class require only very thin or shallow cells. These are made by 'ringing' a slip on the turn-table with one or more coats of gold size or Club enamel. If more than one be needed, the first one must dry before the second is added ; but the final one should only be half dry, or sticky, when the cover is applied. Thin flat objects like a piece of wing need no cement, but will have 'spring' enough to keep them in place. Other objects may require a morsel of gum to fix them to the slip, and in all such cases the gum, and the object also if it has contained any moisture, must be most thoroughly *dry* before covering in, else the moisture will gradually deposit on the cover in small globules invisible to the eye, but too evident when magnified.

Gum cannot be used in this way with very minute objects, such as insect scales or the pollen of flowers. For such, the bottom of the cell may be brushed over with *very dilute* gum, which is allowed to dry into an invisible transparent film. The objects being placed on the surface, very gentle breathing on the slide, or the steam from some hot water, will moisten the gum again, and make the objects adhere. Or they may be affixed to the underside of the cover in the same way. The gum must be *thoroughly* dried again before closing the cell. Gum arabic for slide-mounting should have six drops of glycerine per ounce added, to prevent cracking when dry, and a few drops of some essential oil to avoid mildew; the oil of cloves used in balsam-mounting will answer very well.

The best way to attach scales to the dried gum surface of a slip or cover-glass, is to rub a bit of the wing on the surface. Sufficient will adhere, and can then be fixed as already described. Or, with a hand magnifier or dissecting microscope, a bristle set in a handle may be employed.

When all is thoroughly *dried,* the cover-glass is pressed down on the 'tacky' surface of the varnish ring. The cover should not reach to the outer edge of the ring of varnish. It should if possible be pressed into contact all round. When the ring appears dry, the edge of the cover is ringed round with one or more coats of black varnish or enamel, or with a coat or two of gold-size first, and black varnish over it when dried. The gold-size and varnish should extend a little on the slip, when the varnish cell is very thin, and to the edge of the ring when it is thicker, or when solid cell-walls of any material are used. Such solid cells of paraffined paper or card, tin, glass, or other material, should be cemented on and thoroughly dried before the object is inserted.

Dry *opaque* objects usually require a cell, and most of them look better on a black ground. This may be obtained with transparent slides by using a 'dark well,' as mentioned on p. 104, but opaque cells are often used. A disk of asphalt varnish may be put on by the turn-table, and the cell built up on that. Or on the black varnish may be gummed a disk of dead black paper, and the cell built up over that. And for some objects it is better to bed a cover-glass on a disk of black varnish, before the latter is dry, and gum the object, or objects (such as a group of Foraminifera) upon the glass; the cell and real covering glass being built up over the whole. The cover-glass is put on and sealed as in the preceding paragraph.

**Mounting in Benzol Balsam.**—This is the most durable and generally useful mode of mounting, doing excellent work with entomological subjects, botanical sections, and anatomical sections.

The solution is made by baking Canada balsam on a water-bath, or in a cool oven, till it is as dry as pitch, and then dissolving it in benzol to about the consistency of thick cream. Usually this requires about equal weight of the dry balsam and the fluid. The dry balsam can be purchased, or the solution ready for use. The latter is to be kept in the wide-mouthed capped bottle, shown in fig. 70, with a glass

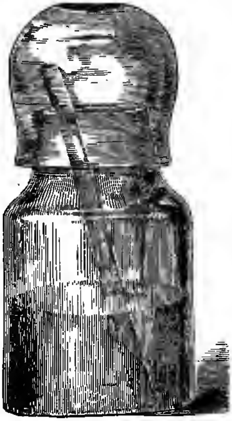

FIG. 70.—Capped Bottle

rod drawn to a blunt point, by which the thick fluid can be dropped upon a slide.

Objects are chiefly mounted in balsam without cells, but simply *pressed* between the slip and cover-glass till dry. If they are too thick for this, as with some hard insects or insect organs, it is usual to employ slips with a concavity ground out of one side. Pressure is maintained either by a small weight, such as a large printer's type, or by one of various patterns of spring clips or compressors, which can be seen at any dealer's shop. Where strong pressure can be borne, common American wooden clothes-pegs answer very well; but much less force is generally advisable.

Insects and parts of insects are best kept till mounting in a bottle of methylated spirit. When wanted, an insect is to be soaked in one or two waters for some hours to remove this, then placed in a solution of one part caustic potash to ten parts of water, to soften and dissolve the fatty parts. This may take a few hours, or it may need days, and must be watched; if overdone, the insect is bleached too much to be of use. When the object is thought soft enough, it is well soaked in several waters to remove the potash, and then treated under water with two camel-hair pencils; one in the left hand, holding down the insect by the thorax; the other with a rolling motion, squeezing out the contents of the body, which are carefully washed away. At this stage, if not convenient to proceed at once, the insect may be kept in acetic acid, which, in that case, must be washed out again with water; but otherwise it may at once be placed on a glass slip and arranged, a slip of paper or thin visiting card laid on each side as a wall or protection, and another glass slip being laid over all; the slips pinching the object are tied tightly together, and put into methylated spirit to get rid of the water; in fact, they should be left in it for hours, and taken out into a saucer of fresh, strong spirit to remove the last traces—really valuable specimens are worth strong alcohol. From this the object is removed into spirit of turpentine, and allowed to soak for a variable time. The turpentine gradually makes the brown, hard, chitinous parts more transparent, and some parts may be the better for days of the process, while other organs only need half an hour. It is still better to place an insect from the spirit first into a small saucer of clove oil to remove the alcohol, and from thence into the turpentine; but this is not absolutely necessary. In all cases the turpentine soak is the final stage before transference to the slide.

The mounting may be done in two ways. One way is to place sufficient balsam solution on the slip with the glass rod, take out the object with a tiny spatula, called a section-lifter, and draining away as much turpentine as possible, work it off the lifter with a needle-point *into* the balsam if possible, or, if needful, another drop of balsam may be placed on top of the object. The cover-glass is then taken with forceps and gently lowered on the balsam from one edge, which will prevent air-bubbles. The cover is then pressed down gently, any extra balsam exuded being removed by blotting-paper, a number of small slips of which are cut and kept ready. When all but enough is taken away the slide is put in a clip and laid away in a warm place to harden; or may be gently warmed for some hours on a brass plate, heated by a lamp, but not enough to cause bubbles.[1]

The other way, strongly advised by Mr. Martin Cole, is to put the balsam on a cleaned cover-glass, and get the object well *into* it; then cover it from dust,[2] and put away for twelve hours. Warm a clean slip, apply a fresh drop of balsam solution to the centre of the partly dried balsam with the object in it, and lay the cover and object carefully down on the warmed slip, avoiding bubbles. Keep the slide warmed a few minutes, and press down rather more strongly. The advantage of this plan is that the balsam is more dried, and the object is nearer the cover-glass.

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[1] Some mounters prefer to heat the slide over a lamp just enough to cause the benzol to boil very gently, the bubbles escaping at the sides. This is all right if the bubbles do not get entangled in the object.

[2] A good way is to invert common tumblers over slides.

Smaller portions of insects, such as legs, antennæ, &c., will need scarcely any soaking in potash, or none, but merely enough in turpentine to make them sufficiently transparent.

Portions like wings need only be dried, then soaked in spirit and finally in turpentine, to avoid air-bubbles.

Sections of stems or vegetable structure may be mounted in the same way; their preparation is briefly described further on.

Balsam slides do not need ringing. When dry, all that is exuded may first be cut round the cover and nearly removed with the tip of a penknife, not going quite close. The slip can then be quite cleaned off with a bit of linen soaked with a few drops of sulphuric ether (keeping this well away from any flame). It can be left so, or the slide very slightly warmed again, to gently melt the edges and make them smooth. Should it be preferred, however, a ring of black varnish or enamel can easily be added on the turn-table.

**Glycerine Jelly Mounts.**—This method also is most simple for the beginner, and one of the most generally useful to the end. It particularly suits small transparent insects, and vegetable subjects, especially mosses and algæ, preserving the colour well. It can be procured in shilling bottles at any microscope optician's. It is a jelly when cold, but liquefies with a gentle heat.

Objects to be mounted in this have to be finally soaked in *water*, or water with a little glycerine mixed in it. Insect subjects therefore only need to be freed from the potash solution; or vegetable tissues to be teased out or dissected in water. The chief thing is to get rid of air-bubbles. It helps this if tiresome subjects are soaked in water that has been well boiled, and finally warmed in it. Insects and vegetable subjects which have been 'treated' as before described, are generally already well soaked with fluid.

The jelly is well kept in a capped glass bottle like fig. 70, and must be liquefied by placing in a cup or basin of hot water. Then, a drop or two being placed on the slide, the object upon that, more jelly over that, and finally the cover-glass gently squeezed down, the whole should be kept warm till all has well settled down, when the mount may be allowed to cool. Superfluous jelly is cut away with a penknife, and the traces left cleaned with a wet camel-hair pencil.

The slides may be finished and sealed by a couple of rings of gold-size, dried successively, and followed by one of black varnish or enamel. But unless the objects (and consequently the layer of jelly) are thick, they will last for years without any sealing at all. My friend, Mr. Henry Scherren, and the Rev. T. R. R. Stebbing, one of the greatest living authorities upon Amphipods, from whom he learnt the method, are in the habit of mounting their smaller specimens in glycerine jelly with no other preservation whatever; and some of these preparations have reached a quite respectable antiquity.

**Protection against Oil.**—Objects which will ever be examined under oil-immersion lenses must however be protected, whatever the medium they are mounted in. This is done by putting on, *outside of anything and everything else*, two successive rings of Hollis's liquid glue, or its equivalent in a solution of shellac in alcohol. This is not acted upon by cedar oil. It is generally best to put on two coats or rings of gold-size before the glue.

**Section Cutting.**—Microtomy or the art of cutting thin sections of tissues has become so exceedingly elaborate that it would be quite impossible to deal with it fully in this work, the serious student will need to consult the *Microtomist's Vade Mecum*. Section cutting of a simple kind is, however, necessary for us to consider, and we must learn how to use one of the simple kinds of microtomes or instruments for sectioning accurately.

The best instrument of this kind for the student is, I think, the Cathcart Darlaston made by Messrs. Watson & Son. This has the great advantage over the ordinary Cathcart that sections of a definite thickness can be cut automatically. It is shown in fig. 71, and the price of the whole instrument in case, fitted for cutting either frozen or embedded sections, is £4 15*s*. Excellent work can be done with this machine, and it will always be useful no matter what other microtomes the worker may acquire later on.

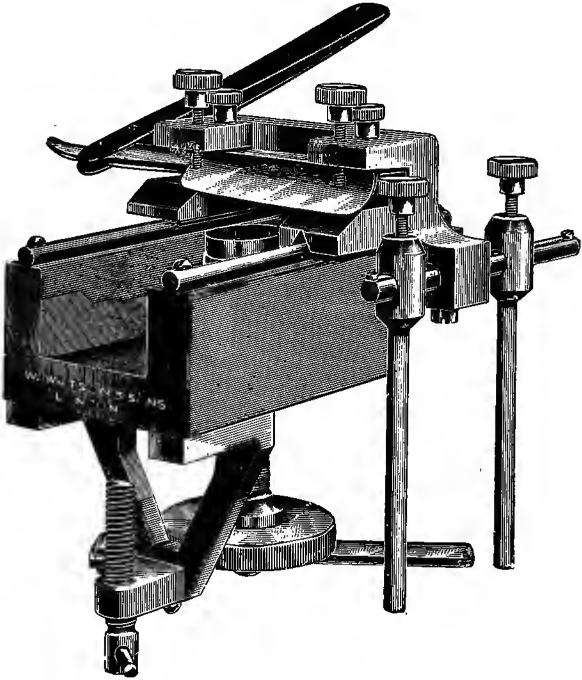


Fig. 71

Wood and stems will need preparation before sectioning. Stems or roots should be soaked for some days in alcohol to dissolve out the resins and then for days in water to dissolve out the gum. Leaves and buds as a rule only need soaking in dilute spirit, or may be cut without any soaking at all.

**Fixing.**—In order that the structure and delicate details of the various cells and tissues may be faithfully preserved it is necessary to kill them as rapidly as possible, and keep them in that condition. This is attained by fixing them in various chemical reagents, the most important of which are—alcohol, bichloride of mercury, acetic acid, formalin, osmic acid and various chromium compounds. The beginner will find such fixatives as formol-salt, Boveri's alcohol, acetic acid, and Zenker's solution the easiest and best for general purposes.

**Fixation in Formol-salt**—This solution is made by diluting formalin with 0·85 per cent. salt solution, the best strength for general cytological work being a 10 per cent. solution of formalin in the salt solution. Portions of tissues are fixed for twenty-four hours or longer in this solution and are then well washed for twelve hours or so in running water. They may then be cut with the freezing microtome or may be passed into 50 per cent. alcohol for embedding as described later.

**Alcohol Acetic Acid.**—This fixative is a most useful all round one for beginners. It is composed of glacial acetic acid one part with absolute alcohol three parts. The addition of one part of chloroform is sometimes of advantage. Tissues are fixed in this for twenty-four hours and are then transferred to 70 per cent. alcohol. They should have several changes of this to remove the acid and then be passed through alcohols of increasing strength, as described later under embedding.

**Cutting Frozen Sections.**—Tissues that require to be cut with the freezing process must be brought into distilled water for some hours. They are then transferred to a syrupy solution of gum acacia in water for an hour and are then ready for cutting. Place a few drops of a solution of sugar in gum water (say gum water five parts, saturated sugar solution three parts) on the plate of the microtome. Work the bellows so as to spray the under surface of the plate with the ether. When the gum solution has frozen, place the tissue to be cut on the mass, add more gum syrup and again work the bellows till frozen, add more syrup, and again freeze till the tissue is covered. Then plane off the sections rapidly. As each one is cut remove it with the finger or a brush to a dish of distilled water. The razor should be kept wet with a drop of dilute gum water.

The best method of mounting such sections on slides is the gelatin method. A 1 or 2 per cent. solution of pure gelatin is made in distilled water. This will keep for a long time if a piece of thymol is added. Glass slides are thoroughly cleaned and laid flat on the table. A drop of the warm gelatin is placed on one end and a very thin film made by touching the drop with the edge of another slide held at an acute angle and drawing it along the slide. The gelatin should spread out into a perfect thin film if the slides are properly clean. As each slide is coated it is stood up on end to dry. When dry store in a rack. For use a slide is taken, gelatin surface upwards (this may be ascertained by breathing on the slide, the coated surface will exhibit colours) and one end is carefully lowered into the dish containing the floating sections. With a glass rod bent at a right angle the water is drawn over the slide and one of the sections floated on, with care, the slide may be withdrawn, leaving the section spread out on the surface. A cigarette paper is wetted and half dried and is then carefully lowered over the section. It is then blotted with blotting paper and the cigarette paper removed by raising it up, starting at the edge. The slide is now at once placed in a corked tube containing a plug of wool soaked in formalin. This should be allowed to act for three minutes, its use is to harden the gelatin and fix the section firmly. On withdrawal, the slide is placed in formol-salt solution where it may remain till required for staining.

**Embedding.**—Tissues must be washed free from fixative, and then transferred to 50 per cent. alcohol for some hours; they then go into 70 per cent., 90 per cent., and absolute alcohol; about three or four hours in each strength, according to the size of the section. They should be transferred to a second lot of absolute alcohol to ensure thorough removal of the water. From thence they are transferred to xylol, or turpentine. They must remain in this until clear. They next go into a mixture of xylol and hard paraffin, and finally into paraffin wax, melting point about 45° to 50° C. They must be kept in a warm chamber whilst in this to ensure the wax is fluid, and should remain in the paraffin at least twelve hours, and preferably more.

They are then cast in the wax, the edges pared down and arranged to go into the holder of the microtome, and the sections cut. The sections are stretched on slides, and the slides placed in a warm place to dry, or they may be pressed on with moist paper.

When dry, the superfluous wax is removed with xylol, the xylol with absolute alcohol, and the slide rapidly passed through alcohols from 90 to 50 per cent. into water. Sections are then ready to stain.

**Staining.** — The art of staining sections cannot be efficiently dealt with in a book of this size and we must therefore content ourselves with describing a few of the most generally useful methods. Those who desire to pursue this further should consult such textbooks as Cross and Coles' *Modern Microscopy*, or Bolles Lee's *Microtomist's Vade Mecum.*

**Ehrlich's Hæmatoxylin.**—This valuable stain is made as follows :—

| | |
|---|---|
| Haematoxylin | 30 grains |
| Absolute alcohol | 3½ fluid ounces |
| Distilled water | 3½ ,, |
| Glycerine | 3 ,, |
| Ammonia alum | 30 grains |
| Glacial acetic acid | 3 drachms |

The hæmatoxylin is dissolved in the alcohol and the alum in the distilled water. To the alum solution add the glycerine and the acetic acid, and then mix with the alcoholic hæmatoxylin, shaking well. The solution should stand for a month to ripen. If required for immediate use, ripening may be accelerated by adding three or four drops of hydrogen peroxide and shaking, when the solution may be used at once.

For use add about eight to ten times the volume of distilled water, or say thirty drops, of the hæmatoxylin to the fluid ounce of water. Sections are stained from fifteen to thirty minutes in this diluted stain, and are then rinsed in distilled water and washed in tap water till blue. They may often be counterstained with eosin with advantage. The eosin is made up to 1 per cent. with distilled water and the sections are stained for one minute in this solution and washed thoroughly again in tap water. They are then dehydrated with absolute alcohol cleared with xylol and mounted in Canada balsam.

**Double Staining in Red and Green.**—This method yields beautiful results with stems, roots and leaves, but is not very useful for animal material. Two stains are required, a red and a green.

RED OR CARMINE STAIN

| | | |
|---|---|---|
| 1. | Carmine | 10 grains |
| | Ammonia | 20 minims |
| | Distilled water | ½ drachm |
| 2. | Borax | 10 grains |
| | Distilled water | 1 ounce |
| | Glycerine | ½ ,, |
| | Alcohol | ½ ,, |

Dissolve the borax in the water, then add the alcohol and the glycerine and mix well. The carmine is dissolved in the water and then the ammonia is added. The two solutions, Nos. 1 and 2, are then mixed and filtered into a clean bottle.

GREEN STAIN

| | |
|---|---|
| Methyl green | 2 grains |
| Distilled water | 3 ounces |
| Glycerine | 1 „ |

For use, stain sections in the methyl green for five to ten minutes and then rinse in water. Stain in the carmine from ten to fifteen minutes. Wash well in absolute alcohol, clear in xylol or clove oil, and mount in balsam.

Most botanical sections will need bleaching before staining. This can be done by immersing in a weak solution of chloride of lime. The best solution to use being:

| | |
|---|---|
| Chloride of lime | 1 ounce |
| Washing soda | 2 ounces |
| Water | 1 pint |

Shake up and allow to stand till clear. Then decant the clear portion for use. After bleaching, the sections are placed in a solution of five drachms sodium sulphite in a pint of water, this gets rid of the chlorine. They are then washed in tap water and are ready for staining.

**Method of Staining Root Tip of Onion as a Test Slide.**—This method is a generally useful one for studying the cytoplasmic inclusions in the cell, especially those known as the mitochondria. A young onion should be selected and placed in a conical glass vessel filled with tap water. The onion should be so arranged that the top is dry and the root fibres hanging free in the liquid. In three or four days a number of fresh rootlets will be found to be growing down into the water. Select several of these and carefully cut them off about a quarter of an inch from the tip. At once place them in the following fixative for twenty-four hours.

Formol 20 c.c., cobalt nitrate 2 grms., sodium chloride 0·8 grm., distilled water 100 c.c.

Next day wash well in tap water for an hour or two, and then transfer to gum syrup and cut very thin frozen sections as already described. Fix on gelatine-coated slides in the ordinary manner. Transfer the slides to a tube containing 2 per cent. chromic +2 per cent. Osmic acid and keep at 50° C. for thirty minutes. Rinse in water, and mordant in iron alum solution, 3 per cent. for six hours, rinse in distilled water and transfer to $\frac{1}{2}$ per cent. hæmatoxylin solution for six hours. Wash in tap water and differentiate in 3 per cent. iron alum, watching the process with the microscope every few minutes. Continue the differentiation till the nuclei show pale grey brown. Transfer to tap water for thirty seconds and then place in 2 per cent. pyridin for two minutes, wash well in tap water for ten minutes. Counterstain in Altmann's acid fuchsin and differentiate in picric acid, transfer to absolute alcohol and then to xylol to clear. Mount in balsam. In successful preparations the nuclei are brownish, the mitochondria red, and the Golgi apparatus black, whilst the cell cytoplasm is pinkish or yellow.

**Charred Sections.**—A method of treating vegetable sections mentioned years ago by Dr. Carpenter, is good and easy, and has been too much neglected. It consists in simply *charring* a section, held between two glasses over a lamp, till it has become charred a dark brown; then mounting plain in benzol balsam.

**Handling Sections.** — What were called *section-lifters* were formerly much used to transfer delicate sections from the final fluid to the glass slip. They consist simply of narrow slips of thin German silver, with one end bent at a convenient angle. The modern, and in most cases better, practice is to take a clean cover in a pair of forceps, and to introduce this under the section in the last fluid used before mounting. Lifters are, however, often necessary in moving from one medium to another in the processes of washing and clearing, and for balsam mounts.

**Storage and Selection of Slides.**—The most economical, safe, and convenient plan of keeping slides is in the pine boxes of trays, as shown in fig. 72. These are sold by scores, of two sizes, holding respectively three dozen and six dozen slides, at about 2*s*. and 3*s*. 6*d*. each. They keep the slides flat and safe at a very small cost, and can be added to just as required. They have the further advantages, that a selection wanted for any occasion can be looked out and put together; and that, as a collection increases, it can be conveniently sorted out and classified. Double trays holding one dozen are also convenient for a few test slides, or to carry a small assortment to a meeting.

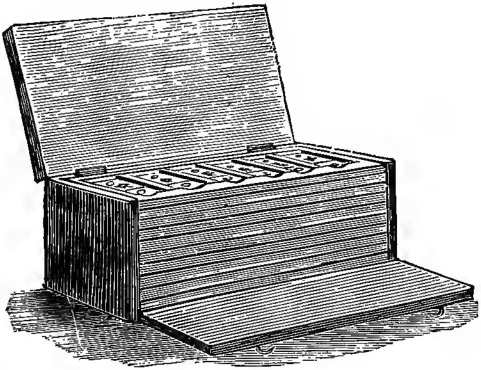


FIG. 72.—Pine-case

The microscopist is by no means dissuaded from buying slides. Such exquisite work is now produced by skilled mounters, that much beauty and interest will be missed by the man who neglects to avail himself of it. But he is strongly advised against idly ordering a 'collection.' It is better to pick up a slide every now and then which excites admiration or arouses interest. Many subjects are advertised from time to time. Bought in this occasional way, a little expenditure gives much more enjoyment, is much less felt, and keeps up the interest, which every purchase will renew as it is made. Slides will also be only purchased when a really *good* one offers; and a much higher average of quality will be secured. The few hundreds of slides which I possess, each chosen with a view to illustrating some occasional lecture, upon the screen, with the lantern microscope, have all been selected in this way, better specimens of some objects being substituted whenever they offered. Thus the very collection becomes a source of interest and gratification, whilst the cost is spread over many years.

## CHAPTER VIII

### POND AND MARINE LIFE

We have now to consider some of the revelations of the microscope. Unfortunately, the space remaining in this volume is quite insufficient for a really scientific classification. I propose, therefore, to divide up this part of our subject into a few groups and merely attempt a very brief survey of each from a general point of view. Thus the chapter following this, although headed Insects, is made to include spiders, which, scientifically, are not insects at all.

I have purposely devoted much more space to this chapter on pond life, for several reasons. It has been my object throughout this little book to try to make students of my readers, and it is very desirable that after the novelty of the instrument has worn off the commencing microscopist should start real work for himself.

There is much work that could be done by amateurs, in this department of microscopic science, that would be of real value to science, and, indeed, in itself it is one of the most interesting branches, for the realm it introduces us to is an entirely new world, the inhabitants of which are without ascertained limits to their minuteness, and whose forms are endlessly varied and beautiful.

Moreover, this study carries the student into the fresh air, and gives him something to do out of doors as well as indoors. It teaches him to look for and look at objects. A great number of the objects now to be described belong to the class termed *Infusoria*, on account of many of them first appearing in infusions of grass or other vegetable matter. Numbers of them may still be obtained in this way, but for the present they will be considered as occurring in ponds and streams only.

The majority of these forms belong to the great division of Protozoa, that is, they belong to the very simplest forms of life. The animal kingdom generally is divided into two great groups, the Protozoa and the Metazoa, and for our purpose we may say that the Protozoa consist of single celled forms, whilst the Metazoa consist of many celled organisms.

Now, as the term 'cell' will be used fairly frequently throughout the remainder of this book, it is desirable that we understand what is meant by the term. Let us examine the slide already referred to, of the growing tip of the onion root. We find that it is made up of a number of more or less oblong bodies, each containing a denser portion N fig. 73 (see frontispiece) called the nucleus. We may, therefore, divide a cell into two portions—a nucleus, and the rest of the cell termed the cytoplasm.

Now, all living things are built up of these bodies termed cells, they are the bricks as it were, with which both animals and plants are fashioned. In the Metazoa, the body is formed of many cells, and the same applies to the higher plants. In the Protozoa, however, the entire organism is formed of a single cell, and we find the same thing in the lowest plants. For purposes of convenience therefore, both the lowest plants and animals are often grouped together under the name of *Protista.* In both we find the entire individual to be represented by a single cell, and the cell consists essentially of nucleus and cytoplasm.

The cells of the body increase by a division into two, each becoming a perfect counterpart of the other. The Protista also increase by a division into two, each becoming an independent organism. This division is initiated within the nucleus, and we must now study the changes the nucleus undergoes during such division.

Let us consider a typical animal cell, such, for instance, as occurs in the skin of the salamander or young newt. We note that in stained preparations, the nucleus consists of numerous dark stained portions surrounded by a membrane, the nuclear membrane. The denser stained portions are formed of a substance termed chromatin, on account of its affinity for basic dyes such as hæmatoxylin. Situated just outside the nucleus in the cytoplasm is a minute dark staining granule termed the centrosome.

As division approaches we find the chromatin becomes arranged into a number of loops, these loops being termed chromosomes. During the formation of the chromosomes, in some cases before, the centrosome splits into two, and the two daughter centrosomes travel to opposite poles of the nucleus. The nuclear membrane disappears, and the centrosomes develop a system of fibres between them. These fibres are known as the attraction fibres, and together form what is known as the spindle. The chromosomes during this process have become V- or U-shaped loops, and become arranged on the equator of the spindle. The loops then split, so that they are doubled in number, and they are seen to be attached to the spindle fibres. They then travel to opposite poles, so that each chromosome at one pole is

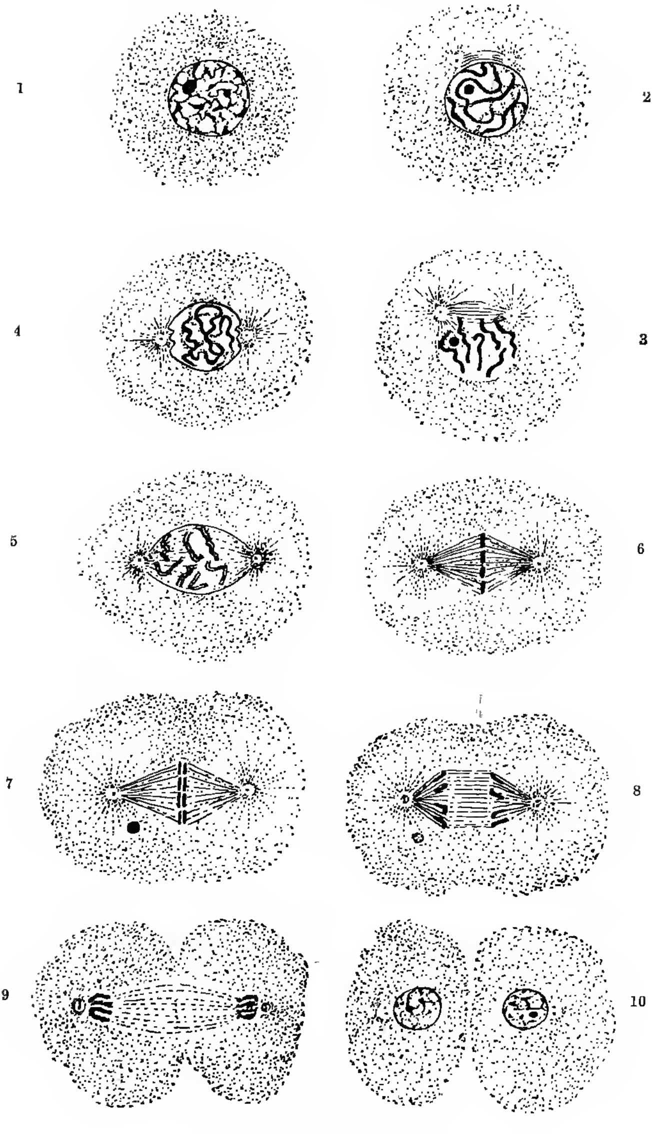


Fig. 74.—Mitotic Division in Cell

1, Resting Nucleus; 2–5, Prophases; 6, 7, Metaphase; 8, Auaphase; 9, 10, Telophase the sister of one at the other pole. Arrived at the pole, they become united again into a tangled skein, and this gradually becomes converted into the typical condition of the ordinary nuclear chromatin. During this process the spindle disappears and a nuclear membrane makes its appearance around each daughter nucleus.

The cell now possesses two nuclei, and division of the cytoplasm occurs by constriction in the middle (fig. 74), so that two cells are formed. It will be seen that this complicated process, which is known as Mitosis, accurately halves the chromatin, and it should be noted that the number of chromosomes is constant for any given species.

Division is not always produced by mitosis, however, and in the Protozoa especially we find almost all types of nuclear division, from a mere splitting of the nucleus into two parts, known as amitotic division, to very perfect mitosis.

**Collection of Pond Life.**—Before setting out to study these minute organisms we must first learn how they are best collected. Mere dipping bottles into a pond and then carrying home the water will lead to disappointment. We must first then consider what apparatus we need and, having got this together, we can then attack any convenient pond or stream.

The first requisite will be some collecting bottles, these should be some three inches long by about one inch wide. They may be provided with corks, which are usually more convenient than stoppers and moreover are cheaper. These can be obtained from any of the leading microscope houses at about 5*s*. per dozen. A metal pocket case to carry half-a-dozen tubes is very convenient and obviates breakages. A suitable case together with tubes can be obtained at most opticians for the sum of 10*s*.

The most useful apparatus for the actual collecting is the net (fig. 75). This consists of a metal ring some six inches in diameter, to which is fastened a net which is made of either mull muslin or fine silk. The bottom of the net is attached by means of cord, or still better, a thick rubber ring, to a flanged glass tube some four inches long and an inch in diameter. Such a net can be easily made by the collector or can be purchased for about 5*s*.

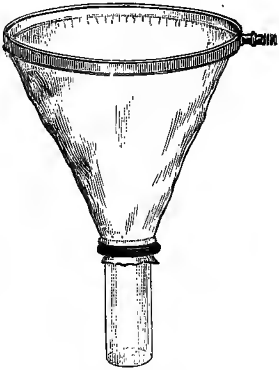


FIG. 75.—Ring, Net, and Tube

The net should be attached to a pole or stick and swept backwards and forwards through the water, either on the surface or deeper down. As the net passes through the water the various organisms are retained within it, whilst the bulk of the water runs through. Finally the ring is turned into the horizontal position and lifted out of the pond. This allows the excess of water to drain away whilst the retained organisms are condensed in the water remaining in the glass tube. The tube is then brought up above the ring and its contents poured into one of the specimen bottles. This bottle should not be filled completely, but a small air space left. It is then to be corked up and labelled with the locality from which the water was obtained.

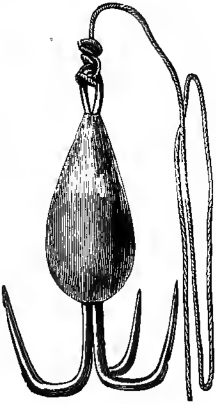


FIG. 76.—Drag

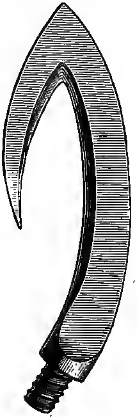


FIG. 77.—Hooked Knife

A drag hook (fig. 76) and a hooked knife (fig. 77) are often useful for dragging up portions of water weeds, as well as sticks and dead plants from the bottom. Many organisms will often be found on these that would be searched for in vain on the surface. A small bottle attached to a stick by a metal collar is useful for obtaining scrapings of mud from the bottom.

All these accessories may be purchased from the dealers, a complete outfit usually consisting of a silver mounted walking stick with net, and cutting knife, drag hook, bottle for mud, and line. The various implements screw into the stick and the whole outfit is very compact. The cost is usually about £1. A good collecting stick is well worth the cost, but when every shilling has to be studied, a little ingenuity can fit a ring to an ordinary stick to carry the net; and the net is the really essential implement. A tow net will be useful for marine work and costs about 18*s*.

Besides the above apparatus the ardent collector will require a good pocket lens such as one of Watson's or Leitz's aplanatic loops giving about × 10 magnification, an additional one of about × 15 or × 20 will also be very useful, and a flat bottle for determining the nature of his catch. A round bottle will do at a pinch, but it is difficult, and at times impossible, to see what one has got unless one uses a bottle with parallel sides. Such a bottle is supplied by most optical firms. But one that answers every purpose may be made by taking a piece of thick rubber about one inch thick and some six inches long by four in breadth. This should be cut to form a kind of U-shaped figure. The sides are next rubbed quite smooth with emery paper and a piece of glass cemented on either side.

The method of using the flat bottle is quite simple. Having filled it up with pond water, some of the weeds or sticks dredged up should be placed therein and carefully examined with the pocket-lens. We shall probably find on many of the stems of water plants, or perhaps on the under surface of leaves, what appears to be a white fluffy substance. This will probably be found to be one of the *Vorticella* or bell animalcules. In the same manner Hydra or the Floscules may be readily identified.

Pond hunting, like most other things worth doing, can only be learnt by experience, and if the student will first master the details given and then sally forth armed for the fray, he will find in a short time that he is making good progress, and is bringing home objects of real interest to study more thoroughly at his leisure. The London student should join the Quekett Microscopical Club, where regular organised pond-hunting excursions are frequent events, and where he may profit by the advice and experience of skilled 'old hands.'

Let us next consider the various organisms likely to be met with in our pond material. As has already been stated, the large majority of these forms belong to the single-celled beings or Protozoa. Protozoa falls naturally into some four main subdivisions or classes, as follows—

1. *Rhizopoda.*—The organisms grouped together under this heading may be briefly defined as Protozoa, which are destitute of any mouth opening, in which the body is naked, and which possess the power of forming extensions of the body protoplasm, termed pseudopodia, or false feet.
2. *Mastigophora.*—This comprises Protozoa, in which the organs of locomotion or of food capture are whip-like appendages called flagella.
3. *Sporozoa.*—This comprises a number of parasitic forms. They are, in the adult condition, without definite organs for locomotion or for ingesting food.
4. *Infusoria.*—The Infusoria are unicellular animals abounding in both fresh and salt water. Their protoplasm possesses a definite limiting membrane, and they possess one or more nuclei. The body is usually pierced by a definite mouth opening. Loco-

motion is generally by means of minute hair-like processes called cilia, or by one or more flagella.

Every living thing, whether animal or vegetable, may be studied from several different points of view, so that in our study of the minute organisms met with in our excursions we must bear this in mind if we wish to obtain a complete history. For our purposes we may consider that these organisms should be studied under some five headings, viz. Morphology, Physiology, Life History, Distribution, and Classification.

**Morphology.**—The first and most obviously important thing which we shall want to study is the form and structure of these wonderful organisms. This constitutes the science of morphology. We must note the general form of the organism and make sketches from different points of view. In no other way can we gain so accurate an idea of the real form and structure of these minute animals. Our studies, however, should not stop here if we have the makings of a real biologist in us.

We must next inquire into the question as to how the various vital functions are carried out and what different parts of the cell are concerned with them. Once we have gained an accurate knowledge of the morphology and physiology of any given form, the next thing we shall desire to study will be what is known as its life history. In the case of the larger animals we can, of course, keep them under observation from birth to the grave, and thus learn their habits, their means of reproduction, and so on. In the case of the Protozoa, however, the whole life cycle is usually gone through in a very much shorter time, and in order to determine it special means are necessary. These will be mentioned at some length as our knowledge of many of the protozoan life cycles is very far from complete, and this is a branch of work that the amateur can be very usefully employed on, the work being of real scientific value.

As nearly all these organisms will be found in water, the first necessity will be some means of keeping a small drop of the water, containing them, under a thin cover glass without drying up. For such a purpose we must use some form of live slide, as it will be necessary to continually observe the forms under high magnifying power.

Perhaps the best slide of this kind is that devised many years ago by the late Dr. Dallinger, and used by him in the determination of the life cycles of certain flagellate organisms. A full description of this slide will be found in *The Microscope*, edited by Dr. Dallinger, p. 342.

A somewhat similar, but simpler one, is that devised by the present writer and Miss Griffin, and described in the *Journal of the Royal Microscopical Society* for February, 1917. This form consists of a glass plate cut to fit the stage of the microscope, with an arm of glass cemented to one side. A piece of linen has a hole cut in it, slightly larger than the cover glass to be used, and this linen, after being damped with water, is laid upon the glass plate. A small glass vessel filled with water is next attached to the arm by means of wire or a rubber ring, and is then put in communication with the linen, either by means of a narrow strip of linen being left on the piece on the stage, or by means of a piece of soft wick. The linen is thus kept moist by capillarity. A drop of the fluid containing the organisms is next put on the glass plate, and a cover glass applied and adjusted so that the circular opening in the linen just touches it at its margin, pressure being regulated if necessary by the insertion of narrow strips of cigarette paper between the slide and the edges of the cover glass. So long as water remains in the vessel attached to the arm, the linen and the fluid under the cover will remain moist. This arrangement permits the continuous use of high-power dry lenses, and usually a water immersion can be employed if necessary.

For many purposes Botterill's live slide can be employed. This may be purchased for a few shillings.

Having procured our apparatus, how shall we set about the study of the life cycle? This is done by patiently following an organism, by means of the mechanical stage if there be one, or by the fingers if not. The great thing is to keep the same form continuously under observation. By such means division may be followed out, and with luck, conjugation, to be described later, may also be studied.

**Distribution.**—We shall be careful to make notes as to localities and exact situations where any given organisms are found; such notes frequently being of much service, particularly where rare or new forms are involved. As regards classification, the serious student will refer to such works as Saville Kent's *Manual of the Infusoria*, or to one of the special treatises on the Protozoa, such as Minchin's *Introduction to the Study of the Protozoa.*

We will next consider some typical organisms from each group likely to be met with, and in order to aid the student in identifying his specimens several plates have been introduced giving sketches of the more usual forms.

**Rhizopoda.**—In examining the mud and vegetable *débris* from ponds, sooner or later, we shall meet with a very remarkable little creature. It looks at first sight like a little, irregularly shaped blob of jelly, with granular contents. The protoplasm may contain numerous diatoms, desmids, and *débris* of all sorts. More careful and accurate examination under a high-power ($\frac{1}{4}$ or better $\frac{1}{6}$-inch objective) will show us several other particulars. Firstly, we shall notice that the protoplasm is divisible into two portions, a clear outer zone, termed the ectoplasm, and a denser central portion, termed the endoplasm. Secondly, we observe that the creature possesses a nucleus, though this is at times difficult to observe in the living state.

If we follow this little creature about for a while we shall see that it possesses the power of locomotion. A portion of the ectoplasm flows slowly out as if it were endeavouring to flow away from the main bulk; this soon ceases, however, and the rest of the organism flows after it, and this is repeated again and again. These extrusions of ectoplasm are termed pseudopodia, and it is by means of the pseudopodia that the organism moves from one spot to another.

They are also actively employed for feeding. In the course of its rambles the organism may come in contact with a small desmid, or perhaps a diatom. When this occurs, we shall see that a pseudopodium flows out and gradually envelops the prey, which is pressed into the soft interior, together with a little of the water surrounding it. It thus comes to be enclosed in a vacuole in the cytoplasm, such a vacuole being termed a food vacuole.

Careful observation will show us that at least one other vacuole is present. This is a clear vacuole, evidently filled with a watery fluid, and, as we watch it, we shall find it will suddenly contract and disappear, expelling its watery contents. It slowly fills again, and again contracts, and this is repeated every now and then. This is known as a 'contractile vacuole' and is common amongst the free living Protozoa. It is to be regarded as an excretionary organ which gets rid of waste products, which would be harmful to the organism.

This minute jelly-like being is called an Amœba, fig. 78, and it constitutes one of the very simplest forms of life. There are many different species of Amœbæ, the majority occurring in water, but some are parasitic, living within the body of the higher animals, usually either in the mouth or intestinal canal. Amœbæ vary greatly in size, some forms being about $\frac{1}{30}$ inch in diameter, whilst others may only measure about $\frac{1}{1000}$ of an inch. One of the largest is very commonly found in ponds and is termed *Amœba proteus*.

Amœbæ are of considerable importance, chiefly owing to the fact that some species are associated with disease. For instance, in tropical dysentery we find an Amœba present in the large bowel in great numbers, and this Amœba, which is known as *Entamœba histolytica*, is the cause of the disease.

Let us next consider the physiology of our Amœba, as much of it will be found to be typical of the whole of the Protozoa. In the first place, we have seen that although destitute of any opening comparable to a mouth, yet the creature feeds actively in the manner already described. We may note in passing that the food is largely other living organisms, and is as complex in structure as the Amœba itself. The water in which the Amœba lives is obviously not pure, and contains various salts which the Amœba probably makes use of.

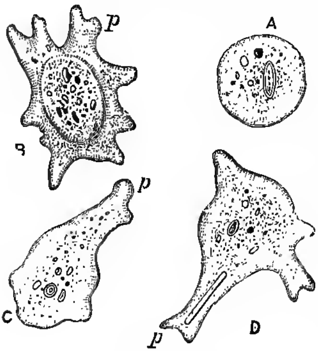


FIG. 78.—Amœba

We have seen that the food when ingested is enclosed in a food vacuole. The cytoplasm secretes a weak acid in this vacuole, and a ferment exists there, probably similar to that found in the gastric juice of the higher animals. This gradually dissolves the food, which, as it becomes soluble, diffuses into the Amœba and is used for building up its own protoplasm. Certain portions of the food, for instance, the silex of the diatom, are left undigested, and these are sooner or later pressed out of the body as waste matter.

Careful experiments have shown that the Amœba absorbs oxygen from the water and gives off carbon dioxide, in other words it respires. Here then we have an organism which uses complex protoplasm as its food, which takes in oxygen and gives off carbon dioxide. Such a type of nutrition is typically animal and is known as Holozoic or animal-like. It is important to bear this in mind, as amongst these microscopic forms it is often very difficult to distinguish plants from animals excepting by their nutrition. Plants cannot generally use complex protoplasm as food, but they build their bodies up from simple salts. Plants moreover absorb carbon dioxide and give off oxygen.

Reproduction takes place in the Amœba by means of fission, the nucleus undergoes division and soon after the protoplasm follows suit. In many cases the full life cycle of the Amœbæ is not accurately known. Fission always occurs, but at times a kind of sexual reproduction may take place, when two individuals fuse together. Such a type of reproduction will be referred to in more detail when we discuss the flagellates.

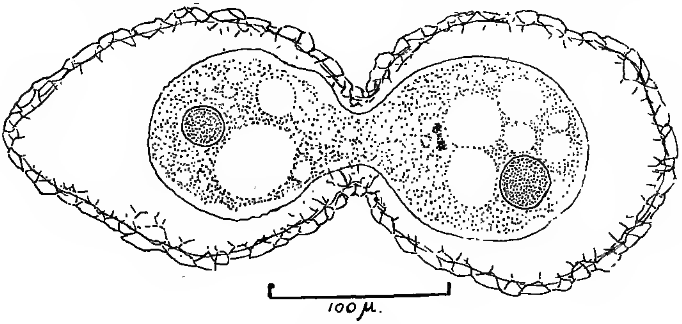


FIG. 79.—Conjugation in Difflugia

**Encystment.**—All the Amœbæ at times form what are termed cysts. The Amœba rounds up and ceases to extrude pseudopodia, a delicate wall appears round the ectoplasm, and this gradually thickens. This cyst wall is very impervious and protects the Amœba from adverse conditions. When conditions again become favourable the cyst wall is dissolved and the Amœba escapes. Many Amœbæ have a flagellate stage in their life cycle, and this should be borne in mind in any detailed study.

We shall probably, sooner or later, come across other organisms belonging to the Rhizopoda, some of these greatly resemble the Amœba in general structure and form of pseudopodia, but are enclosed in a kind of shell or test: this is well seen in Difflugia, fig. 79. The shell in this case is formed of sand and other foreign particles cemented together with a kind of horny secretion. In Actinophrys and Actinospherium (fig. 80) which can with a little search always be found, we have thin pseudopodia sticking out all round, to which food particles readily adhere.

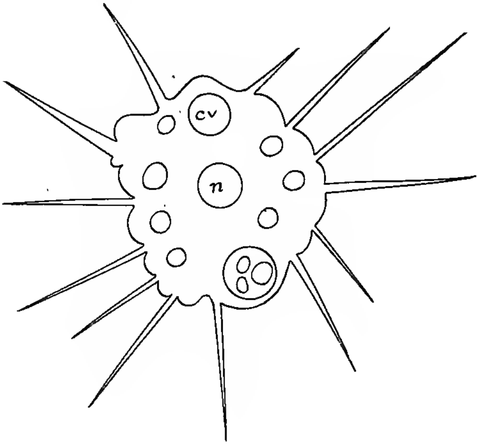


FIG. 80

Somewhat similar forms are the Foraminifera. These have calcareous shells which are pierced with holes through which the pseudopodia are extruded. They are marine in habit. Very similar but having shells of silica are the Polycystina shown in fig. 80A, p. 10. Both these forms exhibit a wonderful variety. Many beds of chalk hundreds of feet thick are formed of the calcareous skeletons of the Foraminifera.

There are two sources of supply ready to the hand of every microscopist. The first is in new sponges. Every one knows the sand that soaks out of a newly purchased sponge. If this is carefully collected and examined in many cases it will be found to contain Foraminifera. The other and better source is the sea shore. With two or three little bags or bottles and a spoon the collector may walk along high water mark and low water mark, and at each level scrape up some of the cleanest and whitest-looking sand left in the ripples caused by the retiring waves in fairly calm weather. Stormy weather mixes up the sand too much and must be avoided. The pocket lens must be used to see if anything is to be got at each spot attempted. Any chance of dredging off shore in ooze, or mud and sand, should also be seized, and the blue clay mud at the mouths of many estuaries will also frequently yield specimens.

The produce of these gatherings has next to be washed and cleaned. First, the salt and mud are washed away in several changes of water. The first gatherings being placed in a large conical glass, and water added, and the whole

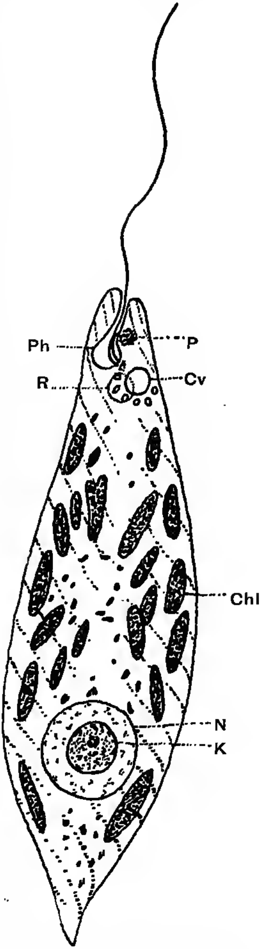


FIG. 81.—Euglena viridis showing Nucleus and Chloroplasts

stirred up and then allowed to settle. The water is then carefully poured off, and more added, and this should be repeated till the water comes away clear. Next, the mass should be strained through two sieves, one of perforated zinc or coarse gauze with meshes or holes about $\frac{1}{8}$ inch in diameter. This will take out weeds and large masses. The other sieve should be of wire or silk gauze and of about 100 to 150 meshes per inch. If still dirty, boil in strong sodium bicarbonate, and then wash all traces of soda away by many changes of water. Finally, dry the remaining mass in the oven and when cold throw into water and stir. The shells being filled with air will mostly float and may be skimmed off the surface while solid sand will sink to the bottom.

The Polycystinæ have, as has already been stated, silicious skeletons: they were named Polycystina by Ehrenberg the great German microscopist, but are now merged in the larger group known as Radiolaria. The classical work on these beautiful organisms is Hæckel's report on the Challenger expedition.

The living forms live chiefly on the surface of the sea, into which the shells sink after death, forming an ooze at the bottom of the ocean. Such ooze, when uplifted as dry land by geological changes, forms silicious sandstone such as that found in Barbados. Should the microscopist come across specimens of the Barbados earth, it may be prepared for examination by breaking up into small pieces and boiling for several hours in a strong solution of ordinary washing soda. This disintegrates the mass, which should be poured off from time to time and the boiling continued till all the pieces are broken up. Allow the disintegrated material to settle and wash as described for the Foraminifera in many changes of water and then allow the Polycystina to settle. Pour off all the water, and scrape up the Polycystina into a test-tube, add some strong nitric acid, and boil cautiously for some ten minutes. Wash away all traces of acid with water and allow the skeletons to settle. Polycystina are best examined on the dark ground.

**Mastigophora.**—The flagellates form a very extensive group, occurring everywhere in fresh and stagnant water and infusions of decaying matter, many forms being marine. Many interesting forms may be obtained by placing animal matter, such as a cod's head, in water and allowing it to putrefy for some days. This was the method adopted by Dallinger and Drysdale in obtaining flagellates for their studies.

The flagellates are classed according to the number, position and proportions of the flagella. Thus we get Monomastigoda, forms with a single flagellum; Paramastigoda, forms with one main flagellum and one or two smaller accessory flagella at the base of the main one; Isomastigoda, forms with from two to four equal flagella; Heteromastigoda, with divergent flagella, one directly forwards or transversely, the other directed backwards; Polymastigoda, with more than four flagella; and lastly Holomastigoda, forms with numerous flagella distributed over the entire surface of the cell. The celebrated Trypanosomes, which are parasites, one of which is the cause of sleeping sickness, belong to the Mastigophora.

With regard to the methods of nutrition this group is very instructive. Thus we get many forms which take in solid food, which they usually capture by the currents created by the vibration of the flagella. Food is often ingested at one particular spot, generally at the base of the flagellum. In such forms the nutrition is obviously holozoic.

Many of these organisms, however, are never seen to ingest food, and they are known to be unable to build up their body protoplasm from salts in the way plants can. How then do they feed? We may picture the water in which such forms abound as containing many of the soluble products of decay, produced by bacteria on animal matter. The flagellate is, as it were, immersed in soup, which it absorbs, not by a mouth, for it has none, but by the whole body. Such a form of nutrition is termed Saprophytic and is very commonly met with.

The majority of the flagellates are very minute, they usually show contractile vacuoles, and possess simple nuclei. One of the commonest met with is *Euglena viridis* (fig. 81). The body in this organism is spindle-shaped and one end is blunter than the other, from it springs a single long flagellum. Euglena swims by means of its flagellum, but it also at times exhibits contraction and expansion, and twists about from side to side. The movements somewhat remind one of a worm, and are so characteristic that the term euglenoid has been applied to them.

Euglena is bright green in colour, the green being due to the presence of chlorophyll. This is the green colouring matter of plants, and it is by its means that the plant is able, with the aid of light, to break up carbon dioxide, retaining the carbon and turning the oxygen adrift. Euglena feeds on solid food, which it captures by the currents set up by the vibration of the long flagellum. This flagellum is set in a depression and food particles are ingested at this particular spot. This is obviously a differentiation of structure and Euglena is therefore more highly organised than Amœba.

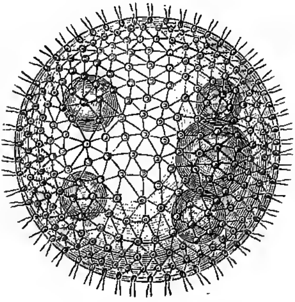


FIG 82.—Volvox × 75

One of the most beautiful of the flagellates is *Volvox globator*, shown in fig. 82. It is commonly found in clear pieces of water, but is very capricious, frequently none may be found in a most likely looking pond, but in small pools close by it may be plentiful. The net will secure it in numbers where a mere dip with a bottle would show none. A well-filled tube appears thickly studded with little green spheres floating about. To see them well place some in one of the thin-fronted troughs and use an inch lens. Volvox is one of the forms for which the tank objectives are very suitable. No black and white drawing can ever give any idea of the real beauty of the organism, which must be seen to be appreciated. It must be noted that each volvox sphere is not a simple animal, but a colony made up of a number of zooids or individuals capable of separate existence. Reproduction is by fission, either into two new daughter forms or at times into many. This method of reproduction by fission may go on continuously for a very long period, but usually some sort of union of two forms, or conjugation, takes place at rare intervals. The result appears to vary greatly.

Fig. 83 shows the life cycle of Mastigella according to Goldschmidt. Here we find the ordinary form dividing by binary fission 2 and 3, and this goes on for a considerable time. Forms sooner or later are found in which minute grains of chromatin are given off from the nucleus, 4a, 5, 5a, 6. In one case these form little rounded bodies which are termed macrogametes and are discharged from the parent cell 9. In the other case flagellated cells are formed, 9a, which are also discharged. These two forms then unite to form a small flagellated individual which divides by ordinary fission (11 and 12) into forms which then grow to the adult stage.

Here we get a difference between the two conjugating gametes, but this does not always exist. For instance, Dobell has described in *Copromonas* a type of conjugation in which the gametes are quite similar to each other. Two forms unite and form a single flagellated form, the nuclei

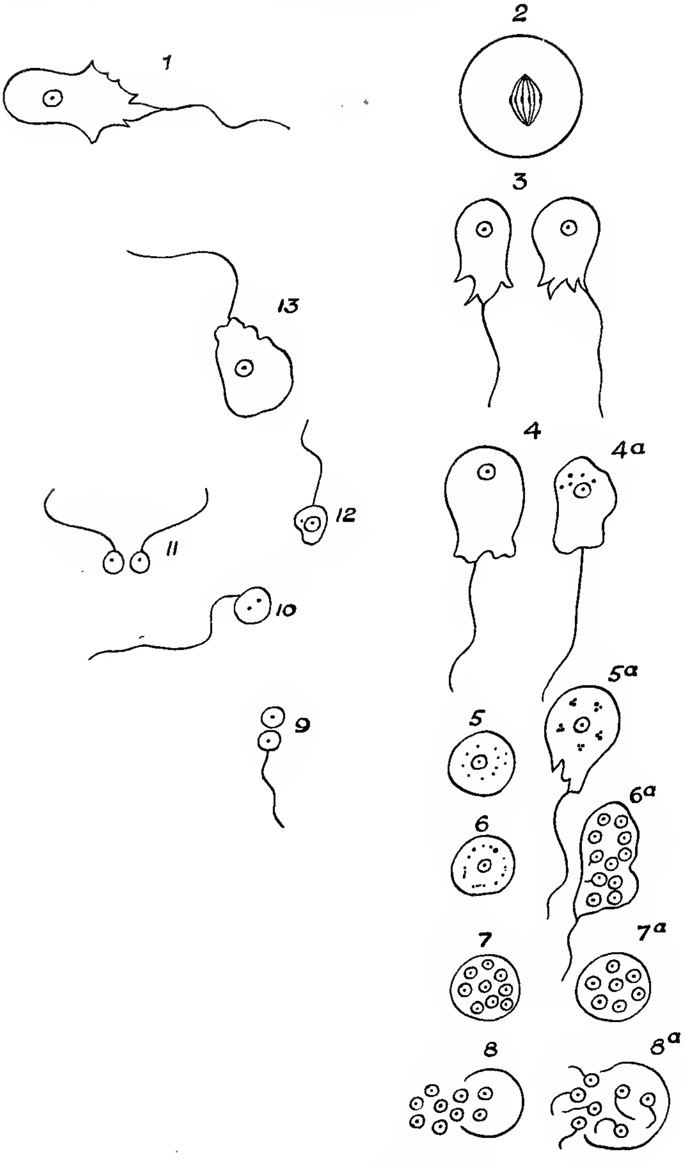


FIG. 83.—Life Cycle of Mastigella

divide, part of the product of division degenerating. Ultimately the remains of the two nuclei unite. The organism may now do one of two things, either it encysts, later on the cyst rupturing and liberating a single adult form, or it may grow to the adult state without encystment. These two life cycles will give an idea of the type that occurs in this group.

Besides being found in collections of water many flagellates are parasites in insects and other forms. These parasitic types often show an alternation of generations being adapted for life in two hosts, and the form inhabiting one host may, and usually is, very different from that found in the other. This is the case with the Trypanosomes which inhabit the digestive track of insects such as the tsetse fly and the blood of animals. For an easily obtained example of the parasitic types we may take Lophomonas which inhabits the intestine of the cockroach. Slides of Trypanosomes can be purchased from the dealers. They usually cost some 2*s*. or 3*s*. each.

**Sporozoa.**—The average student or amateur is not likely to come across the Sporozoa, they are all parasitic forms and will not be dealt with in this book.

**Infusoria.**—Members of the Infusoria abound everywhere in water and they will be the most usual forms met with in our pond huntings. Their forms are extraordinarily diverse, they swim about by means of very minute hair-like processes resembling eyelashes and hence termed cilia. Some have flagella as well.

One of the first which will probably be met with is the beautiful slipper animalcule or *Paramœcium*, and we may as well, therefore, start our studies with a consideration of this form. Its general form can be understood by a reference to fig. 84. It is a long, oval organism, one end being blunter than the other, and has a kind of groove; this groove ends about the middle of the body in a depression. The groove is

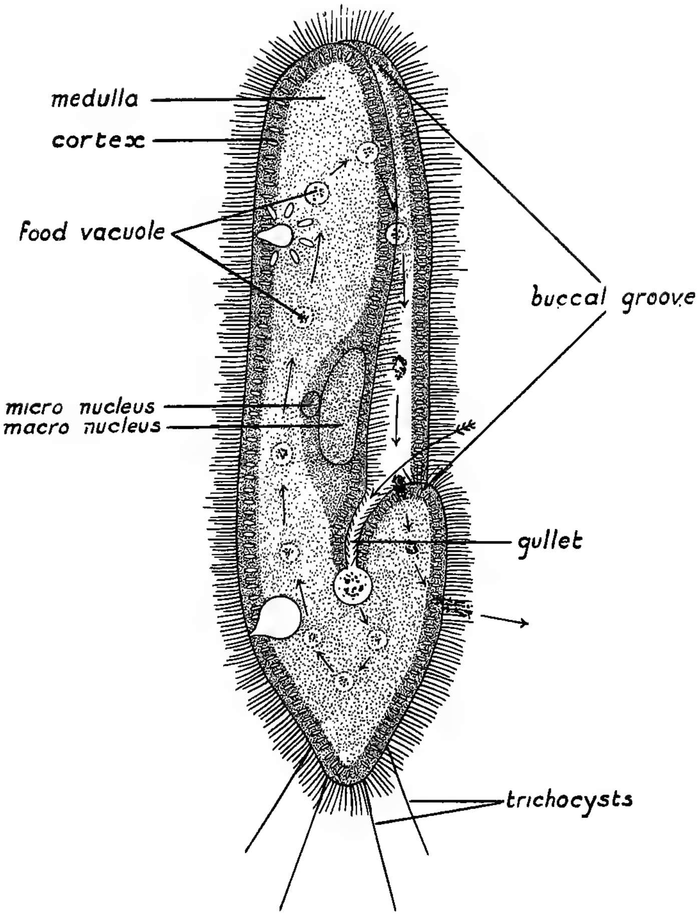


FIG. 84.—Slipper Animalcule or Paramœcium

known as the buccal groove and at its narrow end is a small aperture or mouth leading by means of a short gullet to the internal cytoplasm. The organism is covered with cilia by means of which it swims actively about. The cilia also drive food particles down the buccal groove through the mouth to the interior, where they enter food vacuoles and are rapidly digested.

Staining shows that the animal possesses two nuclei, a large and a small one. The large nucleus is termed the macronucleus whilst the small is the micronucleus. *Paramœcium* multiplies by means of binary fission, but at intervals a kind of sexual reproduction occurs. Two paramœcia come in contact and adhere, the nuclei undergo a rather complicated change and there is an exchange of nuclear material between the two conjugants as they are called. After this the nuclei are reconstituted, the forms separate and actively multiply by fission in the ordinary manner. The essential process in this conjugation appears to be the destruction of the macronucleus and the entire reconstitution of the micronucleus, a fresh macronucleus being formed from the new micronucleus.

If a Paramœcium be killed it will be noticed that long and very delicate threads are sticking out of the ectocyst or outer layer of the cell. These are termed the trichocysts. They are probably of the nature of weapons of offence, similar bodies being found in the Polypes; we shall discuss these later, when considering the Hydra.

Nearly as common as Paramœcium is the swan animalcule known as *Trachelocerca olor*, fig. 85. The long flexible neck is twisted about with great rapidity. The swan animalcules seem to hunt in pairs and when one is seen another is usually close by. *Coleps* or the barrel animalcule is nearly as common. Here the body seems to be sculptured

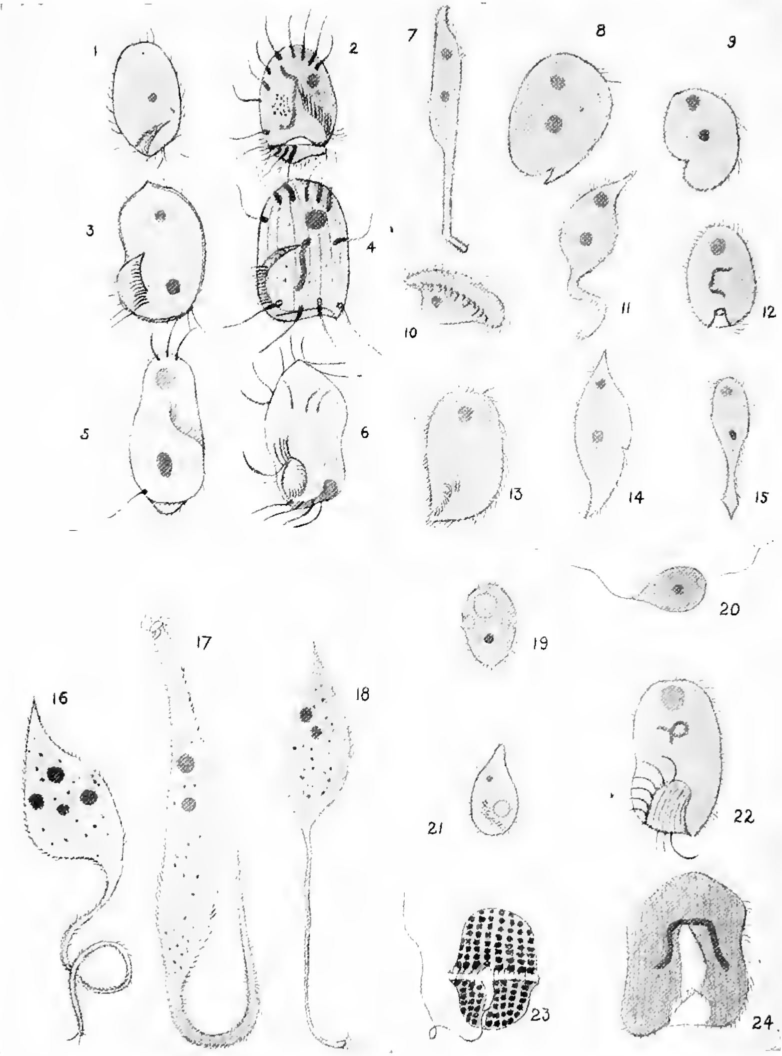
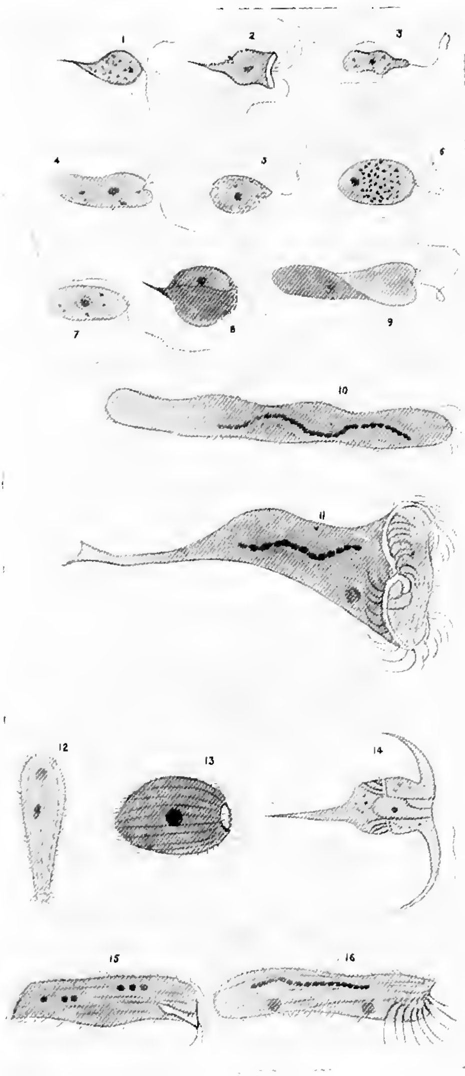


FIG. 85.—1, Oxytricha scutellum; 2, Euplotes patella; 3, Kerona polyporum; 4, Euplotes harpa; 5, Stylonychia mytilus; 6, Psilotricha acuminata; 7, Amphileptus gigas; 8, Trachelius ovum; 9, Colpoda cucullus; 10, Chilodon cucullus (side view); 11, Amphileptus anser; 12, Prorodon niveus; 13, Chilodon cucullus; 14, Amphileptus meleagris; 15, Trachelophyllum apiculatum; 16, Trachelocerca versatalis; 17, Trachelocerca phœnicopterus; 18, Trachelocerca versatalis; 19, Glaucoma scintillans; 20, Heteromita ovata; 21, Panophrys flavicans; 22, Leucophrys patula; 23, Peridinum tabulatum; 24, Bursaria truncatella



1, Spumella vivipara
2, Tetramitus rostratus
3, Dallingeria Drysdali

4, Chilomonas amygdalum
5, Monas Dallingeri
6, Polytoma uvella

7, Bodo saltans
8, Phacus triqueter (red eye-spot)
9, Amblyophis viridis

10, Spirostomum ambiguum

11, Stentor polymorphus

12, Holophrya tarda
13, Plagiopogon coleps
14, Ceratium tripos

15, Condylostoma patens
16, Loxodes rostrum

FIG. 86

into rectangular areas. This form is very voracious. It is sketched in fig. 87.

The bell animalcule or *Vorticella*, fig. 88, is also very common. It is to be found in almost every pond or ditch, frequently attached to water weeds, or sticks and stones at the bottom. In form it resembles a wineglass with a long flexible stem. Let us examine it somewhat more closely. It possesses a contractile vacuole, C.V., a large band-shaped macronucleus, N. 1, and a small micronucleus, N. 2. The arrangement of the cilia is peculiar. The base of the organism expands to a kind of rim, termed the peristome, P. Just within this is a plate-like body called the disc, D. The disc is partly raised at one side. Within the raised portion of the disc is a depression, the mouth M, which leads into the gullet, G. A row of cilia runs

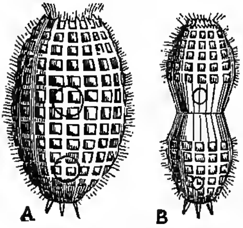


FIG. 87.—Barrel Animalcule
A. Adult B. Dividing

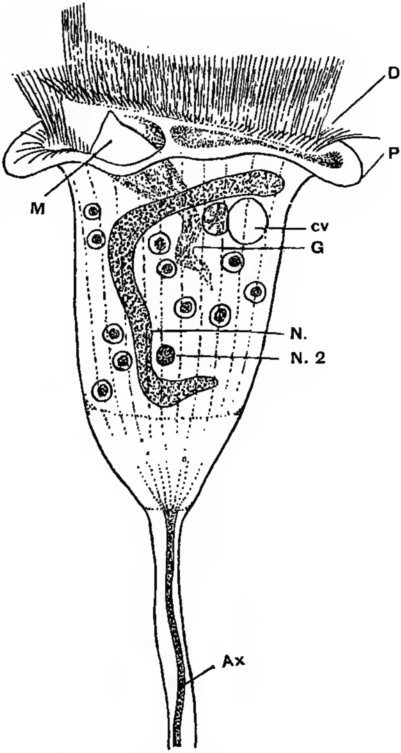


FIG. 88.—Bell Animalcule or Vorticella (showing band-like nucleus, axial fibre in stalk, and expanded peristome)

round the border of the peristome and is continued down the gullet and also round the elevated portion of the disc. The cilia have in consequence a spiral arrangement. The vibration of the cilia give the impression that the disc is actually revolving which of course it does not do. The stalk, s, has a delicate fibre, the axial fibre, A X, running down it, and at the slightest jar of the microscope or anything else which disturbs the *Vorticella*, the stalk is contracted into a spiral, pulling the animal down out of harm's way, whilst the peristome closes up.

*Vorticella* feeds very actively. The vibration of the cilia create currents, and food particles are swept through the mouth into the gullet. This may be shown by introducing a little carmine or indigo into the water, when it will be found to be swept down the gullet and accumulate in food vacuoles in the body cytoplasm.

Reproduction occurs by fission. One of the sister forms remains in possession of the stalk whilst the other develops a wreath of cilia and swims away. After a time it comes to rest, loses its cilia and grows a stalk, and becomes a full-sized *Vorticella.* A conjugation takes place also at times, which is peculiar and interesting. A *Vorticella* divides up, to form either two equal cells or into two that are unequal, one of these divides again to form two to eight small cells, these are termed microzoids; they are somewhat oval bodies and swim away by means of a circlet of cilia. After some time one of these microzoids fuses with one of the ordinary individuals, nuclear alterations take place, and then the organism starts to divide actively again in the ordinary manner. This conjugation in *Vorticella* is interesting, as the two forms are very different in size and origin. The microzoid is regarded as the male element whilst the ordinary form, which is called the macrozoid, is the female.

Besides *Vorticella* we shall meet with colonial forms such as *Carchesium* and *Epistilis*. It is easy to understand how such forms are derived. In *Vorticella* we have seen that after division one form goes free and settles elsewhere, but let us suppose this did not occur. We should get two forms on one stalk, and if division included a portion of the stalk we should ultimately get a branched tree-like colony such as is seen in Carchesium.

Some of the largest animals of the Vorticella type are the Stentors or trumpet animalcules (fig. 89), some of these will be found attached to water weeds and others free swimming. Large numbers of other ciliates will be found from time to time, but space forbids a detailed examination. The plates will, it is hoped, serve to identify many species, and the student will need to consult Saville Kent for anything more he needs. Much useful work may be done in working at the life cycles of the ciliates for in many cases they are very imperfectly known.

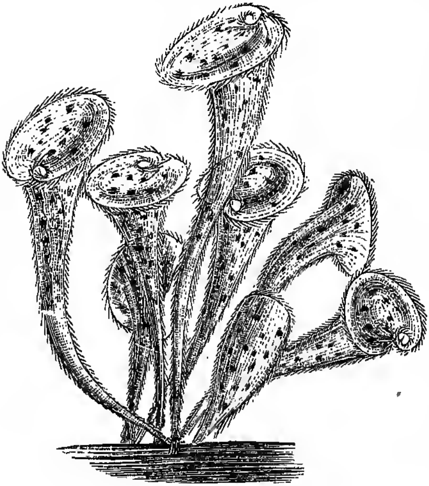


FIG. 89.—Stentor polymorphus × 40

We must look at one more type, generally considered the highest of the Infusoria, inasmuch as the young creature in some genera starts as a Ciliated Infusorian, but afterwards assumes another type. The class is called Tentaculifera, or the Tentacled Infusorians. The example before us is *Acineta*, fig. 90. It is common enough on filaments of confervoid plants more or less loaded with decayed matter, but being only $\frac{1}{100}$th of an inch in height, has to be searched for rather carefully; thus it differs also in being a fixed creature. It cannot be mistaken, being known by its stiff stalk and the triangular shape of the cell, from the outer corners of which can be protruded or drawn in, bunches of minute tentacles, with knobs at their ends. These knobs are suckers, and if one of them touches some small animalcule or other particle of food, others immediately come to assist. But they do not, like the cilia in other forms, sweep down the entire object into the mass below, where it might pass into a vacuole to be digested; they suck out the fluid portion, after which the rest is rejected. As the young are free swimmers, moving freely with the aid of cilia, the growth out of this larval form into another, is a step upward in organisation; and it is remarkable that in the somewhat rare Dendrosoma, many individuals in the adult form unite by their roots in a branched colony, showing also approach towards the Hydrozoa presently mentioned.

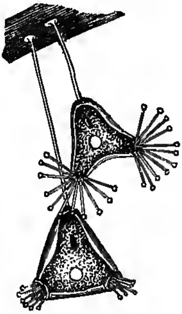


FIG. 90—Acineta

We have now to pass in rapid review some of the other forms of life that will be likely to be found; these are far higher in the scale than are the Protozoa.

The student will frequently find that, in order to study the morphology of his specimens, he must have recourse to fixing and staining processes. For many Protozoa, a drop of dilute iodine solution placed on the edge of the cover-glass and gradually drawn under, with a piece of blotting-paper, will stain the nucleus a faint brown. For the great majority, the following process will enable the student to prepare permanent specimens. Slides are spread with a solution of gelatin, as is described in the section on mounting; the gelatin film should be rather thicker, however. A drop of the infusion containing the organisms is placed on such a slide and very carefully spread out a little. The slide is then put in a tube, film surface upwards. The tube is plugged with moist cotton-wool in order to prevent the preparation drying. After five or ten minutes the slide is withdrawn, and fixed, either by placing in a corked tube containing a bit of wool saturated with 2 per cent. osmic acid solution, in which it remains for five minutes, or by placing in formol vapour. In either case the slides should be transferred to a 5 per cent. solution of formalin for ten minutes to harden the gelatin. In most cases many of the organisms will be found stuck to the gelatin film. Great care must be taken that at no stage does the film dry. After fixation the slides should be washed by placing in a tube of 50 per cent. alcohol for half an hour, they then are passed through 70 per cent. and 90 per cent. alcohol (ten minutes in each), and finally placed in absolute alcohol for fifteen minutes. They are then passed back through the alcohols from 90 per cent. to 50 per cent., a few minutes in each, and are placed in distilled water. The specimens are then ready for staining. The stain may be Ehrlich's hæmatoxylin or carmine, or better, iron hæmatoxylin. For this they are placed in a 2 per cent. iron alum solution for a few hours, rinsed in distilled water, and transferred to a 0·5 per cent. hæmatoxylin solution over night. They are then rinsed in water and differentiated in 2 per cent. iron alum, the process being watched with a microscope and carried on with till the cytoplasm is pale grey, whilst the nucleus is black. When this stage has been reached they are washed in tap water for ten minutes, and are then rapidly passed through 50 per cent., 70 per cent., and 90 per cent. alcohol into absolute alcohol. They are then placed in benzol or xylol to clear, and a drop of balsam and a cover applied.

**Water Fleas and Entomostraca.**—The word *Entomostraca* denotes an 'insect in a shell,' and will be seen to be very appropriate, though it is a curious example of an entirely 'popular' meaning rendered into Greek. The proper water-fleas or *Daphniæ* well deserve their name, from their size, general look, and peculiar jerky motion in the water. There are seven or eight species, which differ in size and shape, but have a strong general resemblance; and they are very common in open ponds which are not very constantly disturbed. So numerous are they sometimes, that clouds of them may be seen in the water in the summer; either

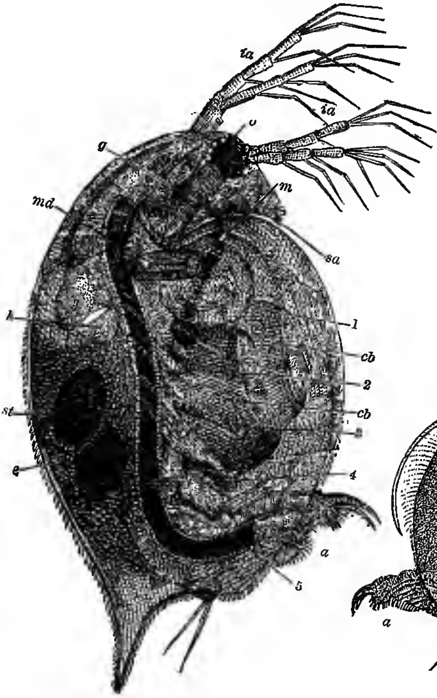


FIG. 91.—Female

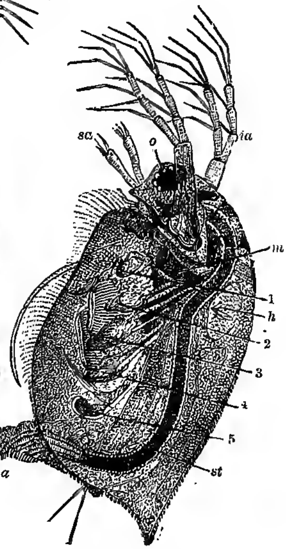


FIG. 91*a*.—Male

whitish clouds like oatmeal, or in other cases of a quite reddish colour. One dip of the net into such a cloud will catch hundreds, sometimes of two or three species; but in almost any pond not much fouled, the net will soon collect some. They are interesting objects, because of their transparency, which allows the digestion, the heart, and blood circulation to be easily seen; and they are easily examined by pushing into a trough just narrow enough to squeeze the enclosing shell *slightly* together, so as to check their motion; or by very slightly squeezing in the live-box.

Fig. 91 represents the female, which is much the largest, and fig. 91*a* the male, of *Daphnia psittaca*, and fig. 92 is a rough diagrammatic section across the body. The shell is not hinged, but joined in one as shown in fig. 92, though it opens and closes to a considerable extent by elasticity at the back. Its outer wall is hard and reticulated, the inner soft, and in the space between globules of blood (*c*, fig. 92) can be seen in motion. In most varieties the shell ends in a pointed spine, and the edges are often serrated or bordered with teeth.

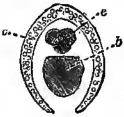


FIG. 92

The head alone of the body is outside the shell. It ends in a beak, which is, however, not a mouth, and its most conspicuous appendages are a pair of superior antennae *sa* (*see* figs. 91, 91*a*) which are near the beak, and very small in the female, and a much larger pair *ia* of inferior antennae, which spring near the neck. These last divide into branches, which are furnished with filaments thickly studded with finest hairs. With these large antennae the creature moves or swims, so that they must be considered a pair of fore limbs.

There is but one eye *o*, though its growth has been traced from two spots, one on each side. It has twenty or more lenses on a black spherical surface, and is shown more clearly at *o*, fig. 93.

Farther back come the real mouth *m* and the mandibles *md*, shown more clearly also in the front view of the head of *D. vetula*, fig. 93. They work from the sides of the head as in insects, and the serrated ends grind together. The *D. vetula* here figured, opens the sides of the shell more than usual, and can often be got on its back so as to show this front view; it is pretty common, and may be known by its nearly round profile and the absence of any spine at the end, as shown in the side view of it, fig. 94. Following the mandibles come a pair of serrated maxillae or jaws, much smaller.

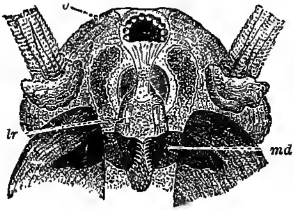


FIG. 93

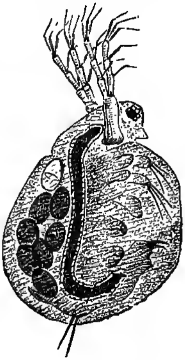


FIG. 94.—D. vetula

The next appendages are interesting, and consist of five pairs of 'legs,' numbered 1, 2, 3, 4, 5, in figs. 91, 91*a*. There is no doubt that they answer to legs in other crustaceans; but the wonderful variety of result, and adaptation of means to ends, of which the Creator's 'natural laws' are capable, is shown in the fact, that whereas in these creatures the antennae have become the locomotive organs, the function of the legs is to assist in feeding and respiration! The first pair may be excepted perhaps from these functions, offering (as seen in fig. 95) the most conspicuous marks of sexual distinction, next to the presence or absence of eggs; for while the first pair in the female have branches or appendages, those of the male are terminated by a hook and single curved filament, which projects beyond the shell as at *f*, fig. 91*a*. The next three pairs are furnished with peculiar comb-like structures (*cb*, fig. 91) (more especially developed in two of the pairs) and shown magnified to a much greater degree in fig. 96, termed the branchial plates. These are in constant motion, and serve to create a current of water up between the sides of the shell. This both conveys infusoria and other particles of food to the mandibles, and air to the gills or breathing organs. At one time it was thought they *were* gills; but the real respiratory structures appear to be certain pouches also attached to the legs, and seen most clearly in fig. 91*a*. in whose walls the blood can with care be seen to circulate rapidly. The hooked 'tail' of the animal, curled round to the front, and often protruded considerably from the shell, is constantly passed up and down between the pairs of legs as if to cleanse them.

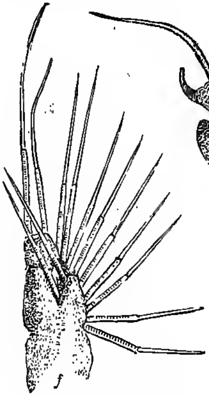


Fig. 95.—Forelegs
*f*. Female *m*. Male

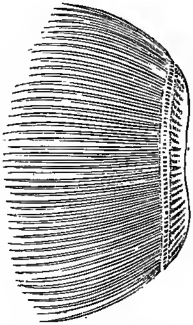


Fig. 96.—Branchial plate

The stomach and intestine *st* are in one, and the contents can easily be seen in motion, until the refuse matter is ejected at the anus *a*. The heart *h* is a structure with a kind of cleft in the middle, which opens and closes in beating, and the whole will be seen to pulsate vigorously.

Passing over many other points of detail, we can only refer in conclusion to the wonderful provision made for the propagation of the species. The body of the animal is only united to the shell about as far down as the heart, and beyond that is quite detached. The eggs are first formed in ovaries at the sides of the stomach-intestine; but are soon transferred into the space between the back of the animal *b* and the shell, as shown at *e* in the diagrams, figs. 92, 97. Here, in the ordinary course, they gradually mature until ready for 'hatching' about the fifth or sixth day, when the young emerge into the water. Their progress can be easily seen in the microscope. This is the ordinary mode of propagation; but it is believed that all such eggs, and probably all the adults, perish in a cold winter, and this has to be provided against. Like larger crustaceans, the Daphnia has at intervals to 'moult' its shell for a larger one. At certain intervals, a thickening of the shell takes place previous to this, at the back, and an extra chamber (*e*, figs. 91 and 97) begins to form outside the ordinary hatching cavity. At first this opens out of the latter, as in fig. 97; but the cavity gradually closes entirely in, with from one to three eggs *e* within it—generally two, forming a sort of saddle (fig. 91, *e*) which is hence called the *ephippium*. The eggs so enclosed, also acquire much more thick and horny shells than the others, and *do not hatch* at the same time. Next moult sees the ephippium thrown off; to float about probably until the spring, when the eggs thus doubly-protected hatch out in the rays of the sun.

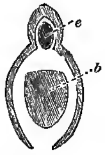


FIG. 97.

Another of the Entomostraca, known as *Cyclops* (fig. 98) is more common even than the *Daphniæ*, being often found in drinking water. Scarcely any piece of water will fail to yield specimens of this, but it does not abound in such multitudes as the preceding. In this creature may be noted the single eye (whence its name) and in most specimens (the great majority being females) the curious pair of bags of eggs dragged about by the parent till ready to hatch; also the curious resemblance in form to the larger Crustaceans. Except to the systematic student, however, there is not so much interesting detail as in the Daphniæ. There is another small crustacean pretty common, the *Cypris* genus, which may be known from its oval *bi-valve* shells.

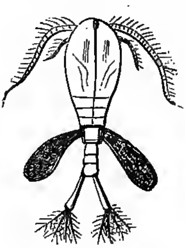


FIG. 98.—Cyclops

**Rotifers.**—In the Vorticella, Stentors, etc., we found advance from unsystematic cilia, to defined circles of them, producing vortices in the water, which swept food into the cavity serving as a mouth. In nearly all pond-water, besides such mere Infusorians, will be found a greater or less number of larger creatures (fig. 99), in which this feature is very clearly demonstrated, and indeed is

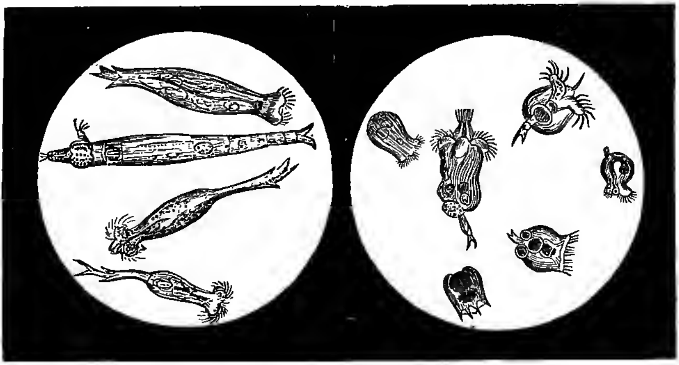


FIG. 99.—Rotifers

the leading character, and which are hence called *Rotifers.* But it is seen almost at the first glance, that they are of much higher organisation. They are of all sizes and shapes, but two special types stand out easily—the long and narrow, and the broader and pitcher-shaped, as shown in fig. 99.

The commonest type of the former is one in the above figure found everywhere, both in salt water and fresh, and known as *Rotifer vulgaris*, a more magnified view of which is given in fig. 100. This was the first to be observed, and has named the whole tribe. It shows at once that Rotifers are *highly-organised* creatures. There are evident signs of *segments*; from which the class is generally held to have some affinity with the worms. The foot with which most are furnished, obviously has toes, and is able to take distinct grasp of fixed points. There is a distinct stomach and intestine; and there are a pair of powerful actual jaws, which work with a machine-like regularity to grind up the food, swept in by the vortex set up by the two sets of cilia. On most genera there are spots which it is thought fulfil at least some of the functions of eyes.

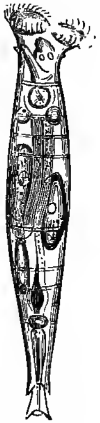


FIG. 100.
R. vulgaris

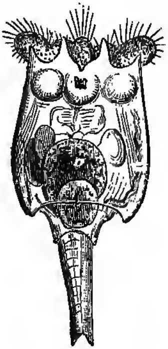


FIG. 101.
Brachionus

Fig. 101 shows a species of *Brachionus*, one of the short and broad type, very common in most ponds. The stem is very flexible, and this Rotifer and many others twist about on the foot in the most grotesque way; or the tail can be quite drawn into the sheath. There are, between the two ciliated discs, tuffs of setæ which do not move while extended.

Amongst the many tribes, there are many more modifications of this kind, which we cannot describe in detail.

A characteristic class of these animals is known as the *tube-dwelling* Rotifers, from the fact that the creature surrounds itself with a case or sheath, either of gelatinous matter secreted, or of pellets built up round it. Fig. 102 shows one of the Floscules. The tube is of transparent jelly, and the crown is divided into lobes which differ in number with the species. From each of these lobes projects a radiating bundle of transparent straight filaments. Below these and the edge of the crown are the cilia. To exhibit the full beauty of a Floscule, will tax the utmost skill of a microscopist. It requires dark-ground illumination; and the more perfectly the optical conditions are adjusted, and the finer the lens, the farther and farther out the long filaments are seen to extend, far beyond what was imagined at first, and of almost inconceivable tenuity.

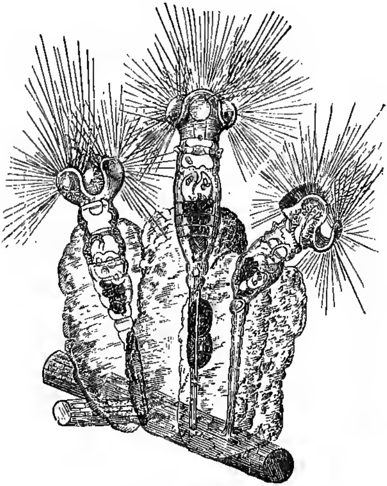


FIG. 102.—Floscules

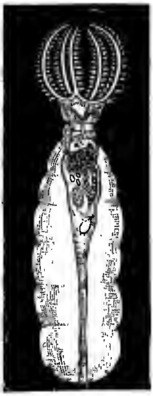


FIG. 103.—Stephanoceros

Allied to the Floscules is *Stephanoceros*, which also secretes a transparent tube. It however is furnished at the top with five curved processes, armed with cilia all the way up, and which spring outwards and then curve inwards again, like the petals of a flower. This is a great favourite, and is also displayed to best advantage on a dark ground. It is generally found on the weed *Anacharis* or the filaments of confervæ, and is very easily seen in a glass tube with the pocket magnifier. Floscules on the other hand are hard to find. I have found both Stephanoceros and the Melicerta next mentioned, breed very freely in the micro-aquarium, and have had long filaments of *Spirogyra* studded thickly with Stephanoceros from end to end.

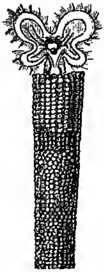


**Fig. 104.** Melicerta

The interesting brick-building Rotifer, *Melicerta ringens*, is easily seen with the naked eye; and few large masses of *Anacharis* can be searched long without finding it. When at work it protrudes four circular lobes with cilia, which look like revolving cog-wheels; but it is very shy, and the least shake to the table makes it drop back into the tube. It looks like a bit of brown pack-thread $\frac{1}{20}$th of an inch long (when fully built up), sticking out perpendicularly from some small leaf or stem, and with apparently the very tiniest morsel of something like thistledown at the top. Under the microscope, the brown tube is seen to be made up of little pellets or bricks: and as close observation is continued, each little pellet can be seen being ground up and formed in the little mill nearly in the centre of the four 'wheels.' When complete the pellet is seized, and the upper portion of the animal bends over and deposits the new brick on the top of the wall it is building. There are some allied varieties; but this is both the commonest and most interesting.

We can only mention in the last place the *clustering* or colonial Rotifers, which are combined in masses bound together by a centre of gelatine, like the Infusoria of similar type (page 172). All are plainly allied, though the shapes of the ciliated crowns differ a little, and also the precise mode of attachment. The most beautiful is perhaps *Conochilus volvox* (fig. 105), in which the individuals are gathered together at the feet by a central lump of jelly, into a spherical ball, much in the fashion of a child's cowslip-ball. The whole group revolves through the water like Volvox (to which of course it is in no way allied), but every separate Rotifer is quite capable of separate free-swimming existence. The drawing is from a beautiful slide mounted by Mr. Rousselet. Both *Megalotrocha* and *Lacinularia* are usually attached to some plant or twig, and are found in running water, whilst *Conochilus* is found in ponds. In *Lacinularia* there is a somewhat large central lump of jelly, (fig. 107) whilst in *Megalotrocha* the feet only are attached and centred at almost a point (fig. 106). The latter may also be known by having four opaque white spots set round the neck, or space under the ciliated corona.

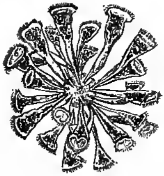


FIG. 105.—Conochilus

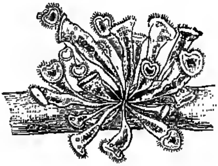


FIG. 106.—Megalotrocha

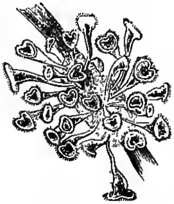


FIG. 107.—Lacinularia

It is interesting to see in these different forms of clustered Rotifers, an evident instance of adaptation to somewhat different conditions. The two last mentioned are capable of revolving in a free condition; and a small new colony often passes some time in that way. It is obvious that in still water this plan of existence offers advantages, both in respiration and finding food; whereas in running water, which of itself brings food and air, the balance of advantage must be in a fixed position.

These clustered Rotifers are seen to the best effect on the dark ground with a low power, needing as they do much 'depth of focus' to see the entire group fairly. They are also beautiful objects treated as opaque, by rays from either the bull's-eye, parabolic reflector, or Lieberkühn.

**Hydrozoa.**—We have in this class of animals quite another type, of which the fresh-water *Hydra* is the general pattern. There are three varieties of Hydra: the common, a browner form, and the green, or *Hydra viridis*, with much shorter and more clumsy tentacles, looking rather like a small budding flower. One or the other can be found in almost any pond where there are plants or duckweed, to which it fixes itself by a sucker at the foot. Fig. 108 shows the Common Hydra; but it differs a great deal in appearance. After a full meal the tentacles are drawn in and blunt: when food has been scarce, the arms may stretch out as thin threads over an inch long. From time to time little buds form as at B, which grow into branching Hydras. There are also eggs at certain seasons.

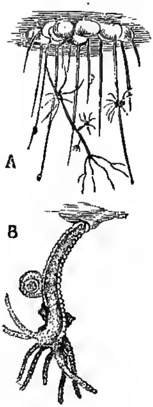


FIG. 108.—Hydra
A. Natural size
B. Enlarged

In one respect the Hydroids are very low and simple animals. There is no stomach or intestine, only a mere bag. On the inside of this are simple ciliated amœboid cells; and when the food has been dragged down into the cavity by the tentacles, particles of it pass into the vacuoles of these to be digested, just as in the lowest forms of life (Infusoria). The stomach is a mere sort of colony of Amœbæ. Hence it is that the Hydra is able to reproduce itself from pieces, as everybody knows; but it is not the fact that the outer skin will do for a stomach if turned inside out, as well as the inside; it is a skin, not furnished with amœba cells, and has to be turned back again if the creature is to live.

But this simple stomach colony is served by a complicated apparatus. Under the microscope the tentacles are seen to be studded with suckers, as shown in B, fig. 108; and if one of these touches a small Rotifer or Infusorian, the latter appears to be paralysed, and in a moment or two is dead. Even Entomostraca are usually killed in a few seconds; but I learnt in a curious way that a strong one, owing to the resisting power of its shell, may sometimes escape. A gentleman told a friend of mine that he was giving a lecture on Pond Life to an audience of soldiers, illustrated by the Lantern Microscope, and a living Hydra was upon the screen, when it grasped a water-flea. The latter, instead of being paralysed as usual, made the most violent efforts to escape, turning over and over, amid excited murmurs from the soldiers, who looked on with intense interest, the power of the Hydra having just previously been explained to them. At last the *Daphnia* hurled itself free; when suddenly every man in the room sprang to his feet and joined in a deafening cheer—the queerest experience, the lecturer said, he ever had!

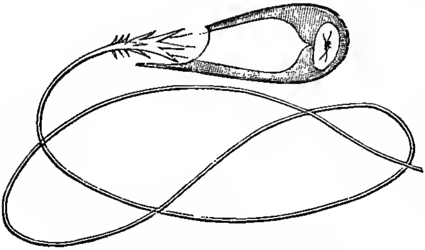


FIG. 109.—Thread cell of Hydra viridis

This deadly effect is produced by 'thread-cells,' with which the tentacles are thickly studded, and the nature of which is shown in fig. 109. Each consists of a little sac or bag filled almost to bursting with a poisonous fluid; and coiled up in the fluid is a long thin filament; the filament as well as the fluid is therefore in a state of tension. Contact or pressure ruptures the sac, when both fluid and filament are forcibly ejected, the end of the filament entering the victim, and piercing it ready for the poison. The small Hydroids are not powerful enough to incommode large animals; but Medusæ or jelly-fishes sting in precisely the same way, and it is well known that their sting amounts to agony. They belong to the same great class as the Hydra; and it is remarkable that many of the Hydrozoa, particularly marine forms, throw off young *which are medusæ*, and swim about in the same way. The thread-cells of the Hydra can generally be made to eject for examination by adding a little magenta or iodine to the water in the trough.

There is another single polyp in the tanks of several Botanic Gardens, which has a beautiful medusa form also: it probably came on some plant from South America. In *Cordylophora,* which Mr. Scherren says is becoming commoner in England, we have a multiple or colonial form, many hydra-like heads branching away from one stem. Marine forms are numerous.

**Polyzoa.**—These are amongst the most beautiful pond subjects. They bear much the same relation to multiple Hydrozoa, as a colony of Rotifers to one of Infusoria: that is, the individuals making up the colony are highly organised, with a stomach and other organs. In mere outward appearance, some marine Polyzoa greatly resemble some Hydroids; but the internal organisation places them upon a higher plane.

Only six fresh-water kinds are often found. *Alcyonella* was once called the fresh-water sponge, and forms in spongy masses round sticks or roots rather deep in the water, in both clear ponds and streams. A small slice cut out will display the beautiful flower-like zooids, with their circular crowns of ciliated tentacles. There are several forms which branch like a tree, *Plumatella* being one of the most beautiful. Two others have each zooid enclosed in a membranous and gelatinous mass. Fig. 110 shows one or two individuals of a colony of *Lophopus crystallinus*, with their crowns of waving tentacles.[1] The most remarkable however in some respects of all the Polyzoa, either in fresh water or salt, is the *Cristatella mucedo*, which when fully developed has an extraordinary resemblance to a thick hairy caterpillar. A single polypide can start a colony, which at first is a small spherical group: but this gradually increases in length. The remarkable point is, that after awhile the entire group assumes a determinate form, of a long oval shape, flat underneath and convex above. The polypides are found arranged in three rows round the back, leaving a space up the middle in which the statoblasts or eggs are developed. And the under side assumes a regular fluted structure, which travels over the pond-weed or twig like the foot of a slug! This is very wonderful and even mysterious; for in some respects the undoubtedly independent individuals appear to be combined

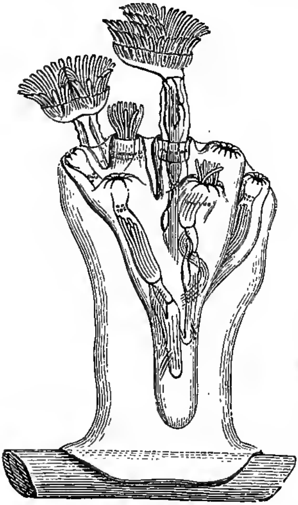


FIG. 110.—Lophopus

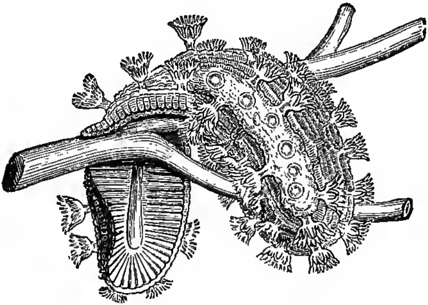


FIG. 111.—Cristatella mucedo (*Allman*) × 5

into a sort of *single animal*, which moves as a whole by common consent, and possesses functional parts (in the foot) which cannot be said to belong to any of the single polypides. This problem cannot be said to be understood. The whole is like a bed of living flowers, and its beauty can be imagined. Fully developed colonies are rare, and chiefly found in autumn in ponds well lit by the sun: the other Polyzoa are mostly found deep, in more or less shady pools. The condenser or spot-lens best brings out their exquisite beauty.

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[1] The drawing is from Allman: but is far too stiff and rigid. There is a flexible *curling* beauty about the crowns, as they are withdrawn or protruded, of which this drawing gives no idea.

**Miscellaneous Pond Life.**—The Desmids, Algæ, and other lowly forms of vegetation are left for Chapter X. Space does not permit us to mention in detail the many objects furnished by larval forms of *Insects* which inhabit the water. The larvæ of various gnats and other small insects are interesting objects. One larva known as *Corethra plumicornis*, or that of the plumed gnat, is as absolutely clear and transparent as glass, and under a low power all the internal organs can be examined. Another beautiful object is the larva of the Mayfly, which is furnished on both sides with many sets of gills, by which oxygen is conveyed to the air-tubes.

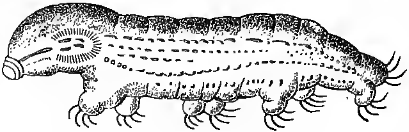

FIG. 112.—Water Bear

Many small worms are also well worth examination. Standing out of the mud will often be seen clusters of red threads waving about: these are the small worms called *Tubifex*. The whole digestive and circulatory apparatus is evident. Shown on the screen by a good lantern microscope, the creature looks like an immense python, gaudily coloured, and ten or more feet long.

There are also interesting varieties of water-mites and water-lice. The most curious of the former is perhaps the very small creature, not larger than some Rotifers, known as the Water Bear. Except that it possesses four legs on each side, it does look ridiculously like a bear, as it crawls about and pokes its bear-like snout into the *débris* on which it feeds.

**Marine Life.**—A harvest of beauty is to be reaped by the seaside, but the ground already gone over will enable us to be very brief. The *net* will be as useful as ever in obtaining small crustaceans, in greater numbers and variety than fresh water affords. It will also obtain plentiful variety of salt-water Rotifers and Infusoria; and on many parts of the coast, skimming of the surface will obtain various marine diatoms. There are also many beautiful forms of minute vegetation, more fully dealt with in the handbook already mentioned.

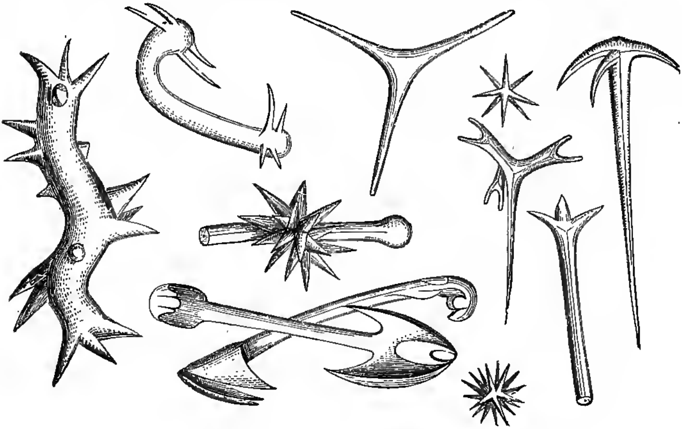

FIG. 113.—Spicules of Sponge

Sponges are a study in themselves. There are one or two fresh-water forms of interest, but the majority are marine. It hardly need be said that sponges are now regarded as colonial animals. Most of them are plentifully furnished with a kind of skeleton of calcareous, or more generally silicious *Spicules*, which of themselves make interesting slides. They may be all joined into one skeleton, as in the beautiful *Euplectella* or Venus' Flower Basket; or they may unite as a looser but connected lattice-work; or (more generally) be detached. The forms vary greatly, from the simplest needles, to types of which a few only are given in fig. 113. Somewhat similar spicules are found amongst Echinoderms, and the 'anchors' of *Synapta* and 'wheels' of *Chirodota* are well-known microscopic objects.

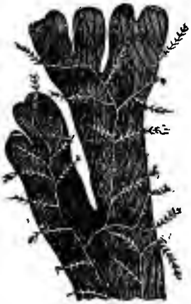

FIG. 114.—Sertularia pumila (*natural size*)

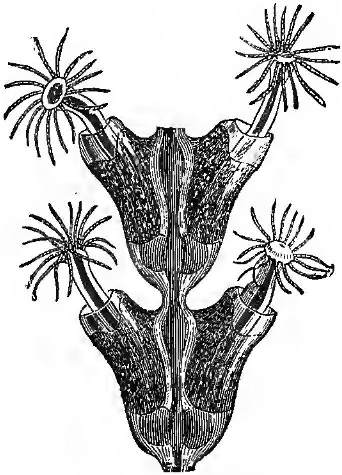

FIG. 115.—S. pumila (*magnified*)

But the greatest variety, and the easiest of the more beautiful subjects to obtain, will be found in the marine Hydrozoa and Polyzoa, which are far more plentiful than the fresh-water forms. Some must be sought in rock-pools, in rather dark cavities, or under the sea-weed: some will be got by the drag-hook (fig. 77) from depths too great to reach otherwise: some are only obtained by dredging from a boat. But a great deal may be gathered from shallow pools, especially the little pools in sand formed by the swish of the tide round points of rock; and many beautiful forms can be obtained by merely turning over the sea-wash, or loose sea-weed thrown up by the tide, whilst still wet and fresh. Let us take only an example or two of what the tyro can be certain to get almost directly.

It is impossible to search long amongst sea-wash without finding a bit of sea-weed with some tiny growth upon it like fig. 114. Place a little bit in a trough, under an inch or two-thirds, and the beautiful Sea-oak Coralline (*Sertularia pumila*, fig. 115) appears, each polypite appearing like a tiny flower, one on each side of each joint of the branching stem. Quite as common in most places is *Coryne fruticosa*, shown of the natural size (*a*) and magnified (*b*) in fig. 116. The tentacles here are seen to be of another type, with globular heads and shorter, and not so free in motion as the preceding. These club-shaped heads are veritable armouries of the stinging 'thread-cells' noticed in the case of the Hydra, and common to the tribe: also there may generally be found some *gonophores* (*c*) or reproductive scions. Great interest will be found in observing these differences, and comparing the polypites, their mode of arrangement on the stem, their grouping &c., which cannot here be indicated in detail. The last of the Hydroids we will mention is the pretty *Lucernaria* (fig. 117). This is usually found on sea-weed or Zostera about (or rather outside of) low-water, and is shown here about the natural size: under the microscope each of the eight arms is seen to have a beautiful tuft of tentacles, which paralyze prey in the usual manner, while between

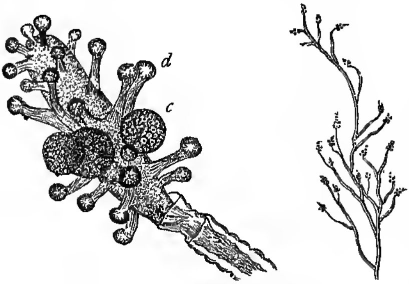

FIG. 116.—Coryne fruticosa

each pair of arms is a smaller organ which serves as a sort of foot or anchor when the creature moves about, as it is constantly doing. This beautiful Hydroid is not so common as the foregoing.

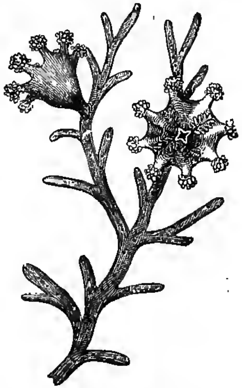

FIG. 117.—Lucernaria

At least one of the marine Polyzoa is as plentiful as the common Hydroids. This is the pretty Nit Coralline. It abides in rather deep water, outside low-water mark as a rule, on sea-weeds there found, and especially on the pretty delicate pink weed itself known as the common Coralline. But I have often found it in sea-wash after a storm. The figure shows the appearance to unaided vision, and when magnified; the spectacle under the microscope being of wonderful beauty. The distinction in character of the Polyzoa will be at once seen, in that amidst a considerable general resemblance, we have obviously a greater importance and complexity of the *individual animal.*

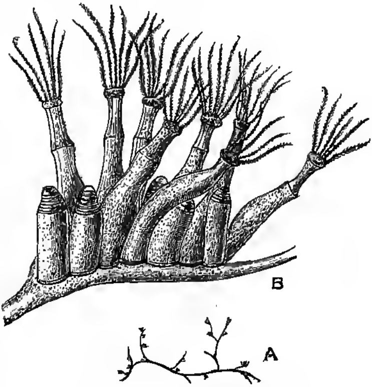

FIG. 118.—Nit Coralline
A. Natural size B. Magnified

Here however we must conclude this chapter, in the hope that some readers may find sufficient interest in Pond and Marine life to follow up the study with more of method and detail.

# CHAPTER IX

## THE INSECT WORLD

NEXT to pond and marine life the Insect World probably opens to the microscopist the most generally attractive, extensive, and easily accessible array of subjects. The ease and facility with which these may be prepared for preservation, has been indicated in Chapter VII.

**The Study of Insects.**—We would specially urge the student to make general observation of the *head and mouth organs of the living insect,* in the manner indicated further on. They are so utterly different from all ideas he may have gained from animals, that in no other way can an intelligent conception of their arrangement, and extraordinary modifications, be attained; and every one who has thus examined a few of the leading types under a low power—from 1 inch to 2 inches—will have acquired an additional range of ideas, and an amount of realistic knowledge, which will impart to his further studies in detail the highest interest.

Our next advice is to carry out microscopic work, as opportunity offers, upon *some definite system.* This will wonderfully add to the interest from the very first; and as the system is carried on, will maintain it to the very last. It may be done in several ways, of which two only need be mentioned as hints for a beginning. We may set out to *study one insect all through,* or at least as regards its more obvious parts and organs. Or we may, on the other hand, set out to study *similar organs* as modified in *different* insects —as for instance wings, legs and feet, mouth organs, &c. It is obvious that the two plans may be happily combined; and that by pursuing either the microscopist will gradually gather a really valuable collection, instead of a mere heterogeneous collection of slides. Let us take an example of each method.

**A House Fly.**—The common house fly—with reference also to the Blow Fly as larger, but otherwise nearly identical in structure—will serve well for the study of an insect, and we can indeed not indicate a tenth of what it offers for study; if anyone desires an idea of *what there is* to study in an insect, let him refer in some library[1] to Mr. Lowne's grand monograph on the Blow Fly. We must take a more general view of the creature, shown as by a strong pocket magnifier in fig. 119.

From this we see its place among *Insecta,* since its many sedgments are plainly 'cut into' three divisions of head, thorax, and abdomen. We notice its apparently single pair of wings, as distinguished from two pairs in many insects: thus classing it among the *Diptera.* We can perceive in general its large compound eyes; and with a little care can see the spots on the sides of its thorax and abdomen, which are the spiracles, or openings of the air-tubes. Its three pairs of legs are plain enough, and mark off true insects from spiders and mites.

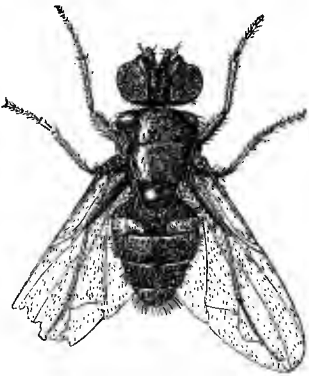

FIG. 119.—House Fly

A very little dissection, also under a low power,[2] will show how totally the *plan* of an insect differs from that of a vertebrate animal. The latter has an internal skeleton: the insect has an outside one of horny substance called *chitine,* to which its muscles are attached. The great nervous chord runs in animals along the back: in insects it is along the front. The animal's circulation is from a central heart through arteries and veins: in insects there are several chambers or hearts, near the back, which drive the blood forward, not through veins, but through the spaces between various organs. And instead of localised lungs or gills, the whole body is traversed by a net-work of air-tubes, with ever so many open entrances along the sides, through which the blood is refreshed with oxygen.

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[1] The book costs, I believe, three guineas.

[2] Insects can be killed for dissection in a wide-mouthed stoppered bottle in which is placed either: (1) Some fresh chloroform on wool. (2) Or some fresh pounded laurel leaves, covered by a piece of perforated card or zinc. A cyanide bottle is more powerful, but somewhat dangerous to have about.

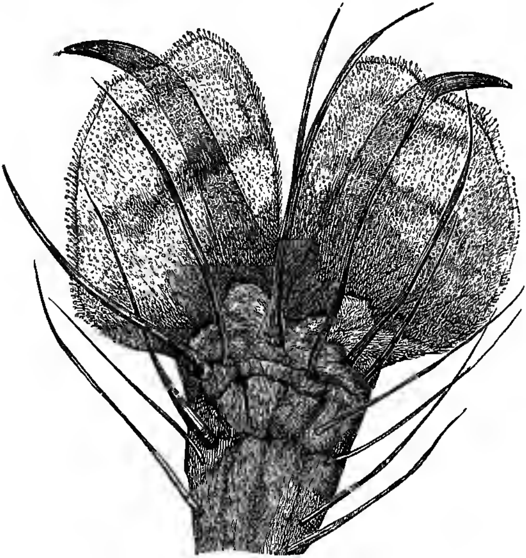


FIG. 120.—Foot of Fly

Let us now examine some of the parts and organs under higher power. And first for the legs and feet. The leg consists of many joints (see p. 195) which are studded with pretty long and coarse hairs. At the foot the most notice-able features are, first, the double claw which is general in nearly all insects; and secondly, the two pads or *pulvilli*, which are by no means so. It is by their means that the fly can walk upon glass; but *how* this is effected can hardly be said to be certain even at the time this is written. For years we used to read that the pads were *suckers* holding on by a vacuum; but it was conclusively shown that this at least was a mistake. Since then it has been generally believed that the small hairs with which the pads are covered, and which appear to have small disks or knobs at the tips, exude a sticky fluid, by which the fly *sticks* on. Though such hairs would let go as the pad was 'rolled' off the surface, it would take a little force to do this; and so in winter, when the flies become feeble, they may often be found stuck by their feet to the window. It is also a fact that they *do* leave a little sticky matter behind them. In 1895, however, Mr. Merlin, using lenses of higher class and wider angle than had been usually employed upon this well-known object, claimed to have discovered that the knobs or disks resolved themselves into very fine curved hairs. His observations have been confirmed by Mr. Nelson and others, and it is possible that in many cases these curved hairs may act as very minute hooklets, in the same way as the caterpillar prolegs described on p. 203. But it is hard to see how adhesion to glass can be obtained in such a manner; and my own observation is that while many hairs, especially round the edges of the pad, can be resolved as described, there are others which cannot be so resolved; and that in the feet of some flies—such as *Asilus* for instance—whose parts are coarser and easier observed, the knobs or disks are unmistakeable. The question cannot be regarded as settled at the time this is written; but the case is mentioned as a good illustration of how much work yet remains to be done, with modern improved apparatus, upon even objects supposed to be well-known to every one.

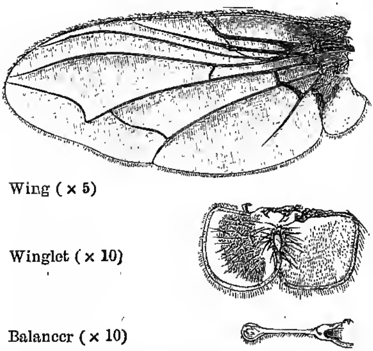


FIG. 121.—Wing of Blow Fly

The wing consists of a double membrane, exquisitely thin and transparent. A moderate power will show that it is thickly studded all over with small hairs. The veining is of course conspicuous, and may be compared with that of other dipterous flies. This is really the fore-wing only. At the base of the posterior edge is a curious double semi-circle called the winglet or *alulet*: when the wing closes one of the semi-circles slides in over the other, and the fore-wing over both. This however is not the rudiment of the posterior wing, nor is its function clearly known: the real hinder wing is represented by the little rod with a knob at the tip, also shown. The pair of these are called *halteres* or balancers. They have some such function: but there are some minute structures and nerves at the thickened base, which will tax the highest powers of the microscope, and which have been thought to signify sense-organs of some sort: here again is further work yet waiting to be done.

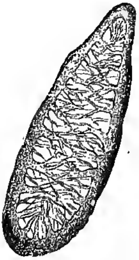


FIG. 122. Spiracle

In the body, we must content ourselves with the respiratory system. A bit of the skin with an air-opening or *spiracle* is easily dissected out and mounted: it is well to take one from both thorax and abdomen. Fig. 122 shows one from the thorax, which is a pretty object, and shows how the opening is guarded by a sort of sieve-like arrangement from the entrance of any coarse particles. Fig. 123

shows a portion of the branched net-work of tubes which communicates with these openings. These tubes have spiral threads of chitine to strengthen them, precisely as wire is coiled inside or outside of rubber tubing for the same purpose, as shown on a larger scale in fig. 124. The structure of these air-tubes is more conspicuously seen in those of larger insects; but a portion of those of our fly can be teased out and mounted without any difficulty.

Turning now to the head, the proboscis (fig. 125) is perhaps the most familiar object in every collection. The figure shows the organ squeezed out flat, which displays well the haired maxillary palpi, *mp*, the powerful muscles and tendons, the fleshy lips *l* with hairs round the margin, the very minute delicate hairs which stud the whole membrane, and whose tips are tests for the very best lenses, and especially the pseudo-tracheæ or sucking-tubes, *tr*. These are guarded by chitinous rings as in the tracheæ, but not of the same structure. There is no continuous spiral; but each ring is detached, and does not go all round, the ends being alternately slightly thickened, and of horseshoe form, as shown at B in fig. 126. It seems probable that fluid can be sucked up through the entire fissure caused by the opening

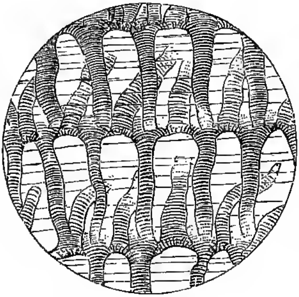


FIG. 123.—Tracheal Tubes

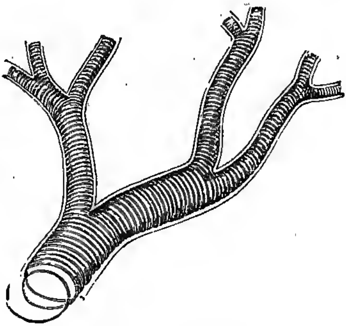


FIG. 124.—Tubes more magnified

between the ends of the rings, which opens on the face of the proboscis; and it is also possible that the hard ridges may have a scraping action: but here again certainty has hardly been obtained.

There are also minute sharp piercing instruments, which are however hidden by this mode of mounting, and are in this fly smaller than in many other Diptera. The proboscis should also be examined from a side-view, preferably without pressure, for the general relation of parts, and for these piercing organs, which are far more developed in some other flies.

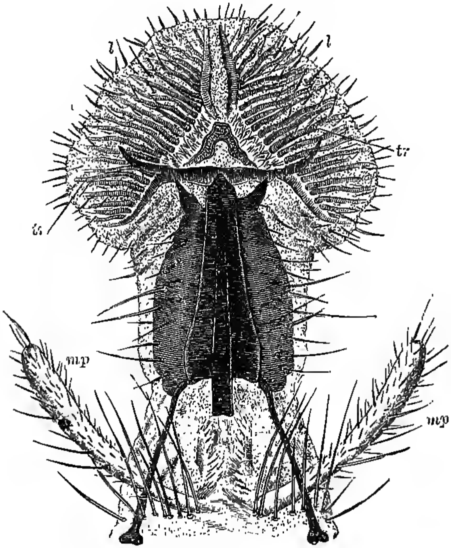


Fig. 125.—Proboscis of Fly

Fig. 127 is the antenna. This has six joints, much less than in many insects; but some of them are too small to be discerned except under the microscope. They are numbered in successive order in the figure. The third joint is peculiar, and will tax the highest powers. Under these the surface is seen to be studded with minute sacs covered with a fine membrane. A somewhat similar structure is far more highly developed in the flattened antennæ of the Cockchafer. It is plain that such delicate organs must minister to some delicate sense; but whether that sense is hearing, smell, feeling, or some extra sense inconceivable to us, is by no means certain—here again is work still to be done.

The *eye* is amongst the most remarkable features. In the case of many insects, the great bulk of the head consists of two immense compound eyes, as may well be seen in the head of a *Tipula* (daddy long-legs). Each mass is seen to consist of thousands of facets, every one of these being a separate lens, focussing the image of the tiny bit of the world immediately in its axis, at the foot of a crystalline cone behind the lens. All rays but a small axial portion are stopped by a diaphragm of black pigment. The general plan of a fly's eye is shown in fig. 128, the nervous rods whose ends take the place of a retina being expanded at their tips near the base of the cones. The precise arrangement differs in various insects. In most flies the lenses have outer convex surfaces; some being plano-convex, and others double-convex. In the eye of the Water-Boatman the

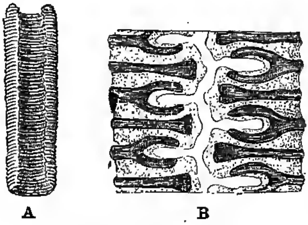


FIG. 126.—Sucking Tubes
A. Tube B. Rings magnified

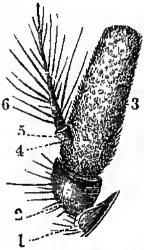


FIG. 127.—Antenna

outer surface is plane, and the inner convex. In the eye of the Cockchafer the lens consists of two parts, an outer lens and an inner one, in contact. The lengths and shapes of the cones also differ.

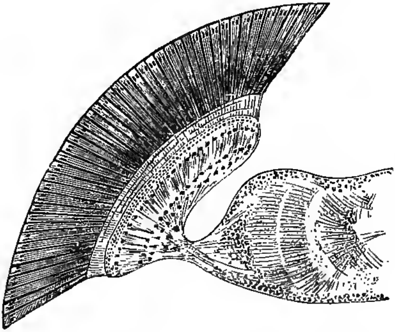


FIG. 128.—Eye of an Insect

To cut good and thin sections of these compound eyes will tax an experienced section-cutter; but it is easy to wash away the rest of the eye from the cornea of lenses, with a camel-hair brush, and mount this cornea flat, snipping the edges a little, that the spherical surface may lie down. The set of lenses of a large insect, such as a water-beetle or dragon-fly, will exhibit the focal power of the lenses, by showing *multiple images* of the source of light. The best plan I find to be to place in the substage (without a condenser, and racked as far back as it will go, close to the mirror) some bright coloured object. Then focus first the cornea itself. On now racking *back* the objective carefully, this will go out of focus, while there will appear many small images of the luminous object. This is a pretty experiment; but it does not convey a true idea of insect vision, or of anything at all beyond the fact that these tiny lenses *are* real lenses. It has been shown by actual experiment, that when an insect's compound eye is used to take an *actual photograph* in its natural spherical condition (which has been done by Professor Exner) there are not many small images, but that one large image is truly pictured.

Besides these compound eyes, many insects have several *single* eyes on the top of the head, between them. Three is the most usual number, and these are set in a triangular form. In our fly they are very small; in wasps and drone-flies they are conspicuous. The relation of these single eyes to the others, and to the total vision of the insect, is at present a great mystery.

Here we must leave our fly, with much about it not even alluded to. There are the digestive system, the muscles the nerves, and many other details which will all repay study; but we must pass to another point of view. Let us now suppose that the student desires to make comparisons, and study resemblances and differences. As we began about the fly with its feet, let us take for example of this method, a few specimens from the legs and feet of other insects.

**Legs and Feet of Insects.**—First take a couple of larval forms, one quite general, the other much the reverse. Fig. 121 represents the feet of the Goat-moth caterpillar, a general type of many other caterpillars. The top figure is the end of one of the 'pro-legs,' which are mere membranous projections from the skin, a pair springing from each of four segments, and another pair from the last segment of all. The end or foot is furnished with a row of recurved hooks all round; and it is the hold of these hooklets upon the skin, which causes the 'creepy' feeling when a caterpillar crawls on the hand. All these 'legs' totally disappear with the larval stage; and the three pairs of real legs are represented by the short-jointed organ, with a single claw, shown in the lower part of the figure.

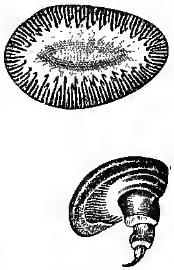


FIG. 129

Not legs in the proper sense, but certainly locomotive organs of a peculiar kind, are shown in fig. 122, the very minute larva (magnified) of *Tanypus maculatus*, a small twowinged fly, found in the flocculent vegetable matter at the bottom of ponds. It is very transparent, and a beautiful object; but we have only to do with the peculiar organs shown, projecting from a segment behind the head, and at the 'tail.' At first nothing may be visible; but gradually a little projection comes out, then this forks into two, and finally from the end of each starts a mass of little hooklets, as shown in the upper of the more magnified figures. In the other fork these are shown drawn in, as the whole can be at will by the muscle seen passing down the centre of the branch. The pair at the tail are very similar, as shown in the other enlarged figure.

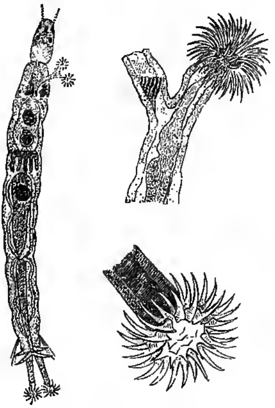


FIG. 130

Going now to adult insects, a normal example will be the leg of a cockroach, which shows the five parts. First is the *coxa*, *a*, next a small joint called the *trochanter*, *b*, next the thigh or *femur*, *c*, then the *tibia*, *d*, and finally the *tarsus*, *e*, here and normally with six joints and ending in a double claw, which ends the limb in the great majority of cases. Leaving the host of beetles and other insects which have legs closely adherent to this type, we will look at some curious developments and modifications.

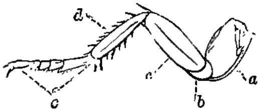


FIG. 131.—Cockroach leg

Perhaps the most striking are amongst the water-beetles. Fig. 132 is the foot of the fore-leg of the male of the great water-beetle, or *Dyticus marginalis*. Here the first three joints of the tarsus are expanded into a broad surface, which is fringed all round with curved hairs. From the surface itself spring a great number of short hairs with cup-shaped disks at their extremities; the greater number small, but one very large indeed, and another (in some varieties two others) of intermediate size. All have practically the same general structure, as shown in the more magnified view given in fig. 133. Ridges of chitine go from centre to circumference of the cups, and the idea of their being 'suckers' is almost irresistible; but it has been all but proved that they are not, but simply apparatus for conveying a viscid adhesive fluid, and in fact a further development of the hairs mentioned in describing the foot of the fly, page 197. The stem of the large 'sucker' is easily overlooked unless looked for and examined sideways. The other two joints are terminated by a double claw, in this case unusually large. The hind legs of the same insect are also worth mounting, for their immense fringe of long and thick hairs, which act as a propelling paddle when expanded.

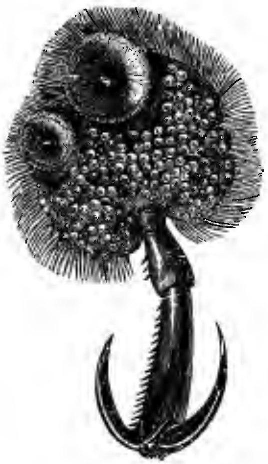


FIG. 132.—Tarsus of Dyticus

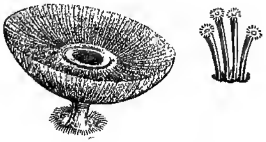


FIG. 133.—Hairs with Disks

Our next example is the expanding paddle of the *Gyrinus* or Whirling beetle. The front pair of legs are of the ordinary type, but the hinder pairs are peculiar (fig. 134). The trochanter *tr*, femur *fem*, and tibia (*tib*) are flat plates of triangular shape, jointed at outer angles, from which the apex of each next plate springs. But the tarsus (*tar*) is jointed on at the *inner* angle of the outer end of the tibia, and each of its four joints is expanded on one side into a flat paddle - blade. These are shown nearly closed in the upper figure, and expanded and more enlarged in the lower, where the joints of the tarsus are numbered. The paddles are moreover fringed with flat hairs which further increase the surface. The fourth joint bears a small fifth one with its usual double hook.

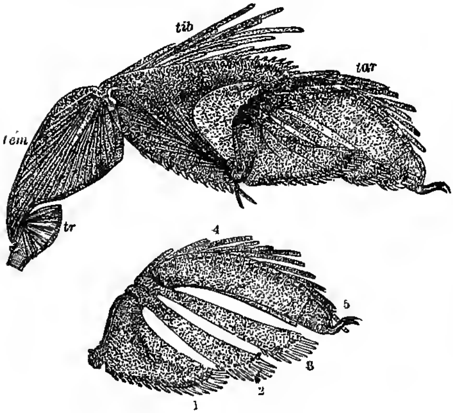


FIG. 134.—Paddle of Gyrinus

Fig. 135 is the foreleg of the Water Scorpion. Here the femur *f* becomes by far the largest joint, containing muscles of comparatively tremendous power. These muscles close the tibia like the blade of a knife upon the femur, which is grooved like the handle of the knife to receive it. The claw alone here represents the tarsus *tar*, and is not jointed. This powerful instrument is employed by the insect to seize and crush its prey.

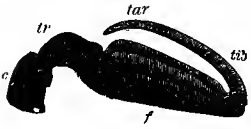


FIG. 135

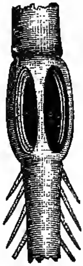


FIG. 136

Fig. 136 shows the tibia of the foreleg of the Great Green Grasshopper, which is remarkable for the pair of orifices, oval in shape, near the upper end. These orifices are in direct communication with the main tracheal tubes which pass down the limb. Such a remarkable feature must have some special function; but this has not yet been discovered. It is possible that other instances of the same kind might be observed if search were made for them.

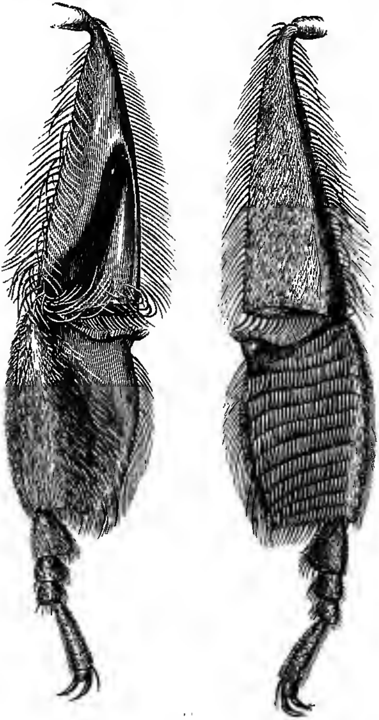


FIG. 137.—Leg of Worker Bee

Many interesting variations may be found in the legs and feet of flies, Lepidoptera, and other orders; but we must only further notice the hind-legs of the worker-bee. Here both the tibia and the first joint of the tarsus are broadened into wide plates, but the two sides of these plates are differently furnished. On one side they are rather hollowed out and comparatively bare; and in these hollows are carried home the pollen-bread and the resinous *propolis* used in finishing and cementing cells. On the other side are a thick coating of hairs, those on the tarsus taking the form of a brush, which appears to be used in collecting pollen. Neither of these special developments are found on the hind-legs of the drones or of the queens.

So much for examples of quite different systems—but still *systems*—for the microscopical study of the insect world: we must content ourselves further with a few miscellaneous subjects of interest amongst insects and spiders.

**Wings of Insects.**—These offer very interesting and easy subjects for the 'comparative' method, all the more instructive because upon them the great insect orders were originally, and are still mainly founded. The Orthoptera were the 'straight' wings, the Neuroptera the 'nerved' wings, the Trichoptera the 'hairy' wings, the Coleoptera the 'cased' or 'sheathed' wings, the Diptera the 'two' wings, the Hymenoptera the 'married' wings, the Lepidoptera the 'scaled' wings.

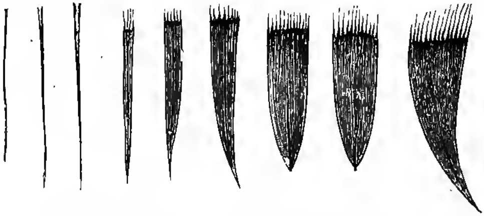
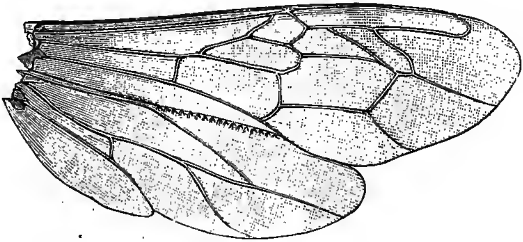


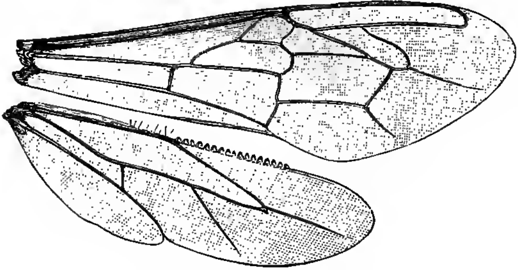
FIG. 138.—Scales from Convolvulus Hawk-moth (× 20)

In all the orders there have been found most interesting variations, especially in such wings as are very minute. Some are best mounted dry: for instance the wing of the Hairy Midge (*Psychoda*) is apt to lose the hairs with which it is loaded if immersed in fluid—they come off so easily. And the smaller membranous wings are so transparent that some of them are really better as dry mounts. But the majority mount in benzol-balsam quite well. The thick horny cases of many beetle wings need to be made transparent by long soaking, in the manner already described (Chap. VII). The elytra or wing-cases of iridescent beetles make beautiful objects mounted opaque for the Lieberkühn or reflector, and so do bits of wing from the iridescent and bright-coloured butterflies and moths.

Whole wings of the latter mount well in balsam. It is very easy to mount the whole or a piece of a wing to show the arrangement of the scales. Some wings will show the scales so sparse that they stand clearly apart, and in the clear-winged moths the scales are rare, and more or less interspersed with hairs.



In flight



Separated

FIG. 139.—Wings of Bee (× 7)

A *scale* is in fact only a peculiar development of a *hair*, and the particular development varies amazingly. Scales rubbed off from different genera, and even species, and mounted *dry*, will make an interesting set of slides. Also from the Convolvulus Hawk-moth (and many others) *sets* of scales may be so selected and mounted as to illustrate clearly the gradual development of hairs into scales. Fig. 138 is drawn from a set of scales I bad so mounted, all of which were taken from the same individual insect, and show the transition clearly. Again, it will be found that the membranous wings of all insects are covered with small hairs; and if the wing of a Gnat be carefully mounted, it will show that on many portions, especially the veins and the edges, these hairs have become scales, in no essential respect differing from butterfly scales.

Fig. 139 shows the Hymenopterous or 'married' type of wings. The two are also shown separated, to manifest on the hinder wing the row of little hooks standing up, and on the posterior edge of the fore-wing, the turned-down edge into which the hooks catch. Thus, when in position, the two are joined together. It will be found very interesting to trace this character running not only through the Bees and Wasps, but through such totally different-looking groups of insects as the Ichneumons, Saw-flies, Gall-flies, and Ants, even of small size.

**Mouth Organs.**—Similarly interesting and important are the mouth organs, equally important in classifying with the wings, and sometimes more so. For a primitive and normal type take the Cockroach (fig. 140). The main portions of the apparatus are here shown separated. With care the mouth-parts of insects can be so separated, and laid down upon the slide, arranging them in benzol-balsam, and leaving the preparation to nearly dry in the balsam, protected from dust, before laying down the cover-glass. Such a preparation is known as the *trophi* of an insect, and a set of trophi, of various species throughout the orders, is a most valuable and instructive set of slides, such as cannot be bought for money anywhere.[1]

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[1] A couple of dozen I possess, kindly mounted for me by the late Mr. Tatem, of Reading, are among the most valued slides in my collection.

Looking now at the cockroach's mouth, the whole apparatus is a complete upset of all our vertebrate notions, though in many respects similar to that of crustacea. There is a sort of roof or upper lip *a* called the *labrum.* Next come the first and principal pair of jaws *b*, the mandibles, but which work from *side to side*, as do all the organs, and not like ours. These are hard and strong and toothed. Below these are a second pair less strong, but toothed also, *c*, called the *maxillæ*, and each of which has a *palp* or feeler, but which is more really a sort of hand to help in manipulating things. Beneath all is the lower lip or *labium*, *e*, but which under higher power is made out to be essentially a third, but still less powerful pair of maxillæ, each of which also has a palp. In the cavity of the mouth is a *lingua d*, which is something like a real tongue, receiving the duct of the salivary glands.

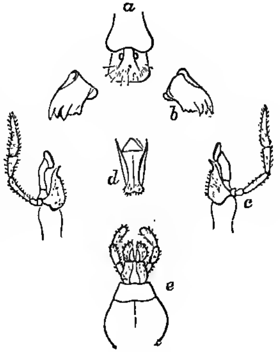

FIG. 140

Leaving this ancient type, it will be most interesting to see how in the main it is preserved in all the Beetles or Coleoptera, with chiefly the more or less complete disappearance of jaw-like character from the parts of the *labium*, or third pair. Next to be modified are the *second* pair, the maxillæ. It will be found of great interest to follow up, with the help of some really intelligent work on entomology, the transformations of these organs and their palpi, with those of the *labium*, into the 'tongue' of bees and wasps, the 'proboscis' of moths and butterflies, and so on; the mandibles either remaining as biting jaws—as in the bee—or being reduced to rudiments, as in butterflies. We take two examples from a different order again, the Diptera, in which these organs

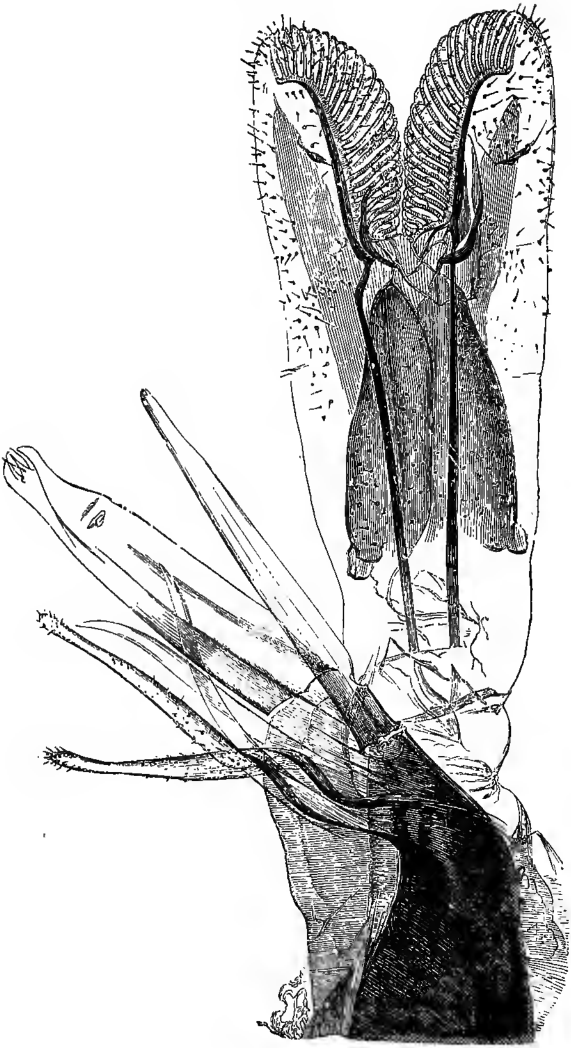

Fig. 141.—Mouth Organs of Drone Fly (× 25)

are so modified as to assume more or less of the character of *weapons.* Fig. 141 shows those of the Drone-fly, taken first as resembling in many respects those of the common fly already mentioned (fig. 125). The soft parts of the proboscis, with their pseudo-tracheal tubes, are almost precisely similar, as will be seen, and represent the anterior part of the labium. The labrum, mandibles, and maxillæ are in the Diptera converted into lancet-shaped organs more or less developed, which both pierce the skin of animals, and form tubes by which the juices may be sucked up. In the Drone-fly they are much larger than in the House-fly, and easily made visible. The figure shows clearly the maxillary palpi, next which come a couple of lancets, and finally two instruments resembling by comparison straight two-edged swords, and a very peculiar one with prongs or teeth at the end, the purpose of which appears to be to enlarge and irritate a wound and so increase the flow of fluid, which is probably sucked through a tube up the centre.

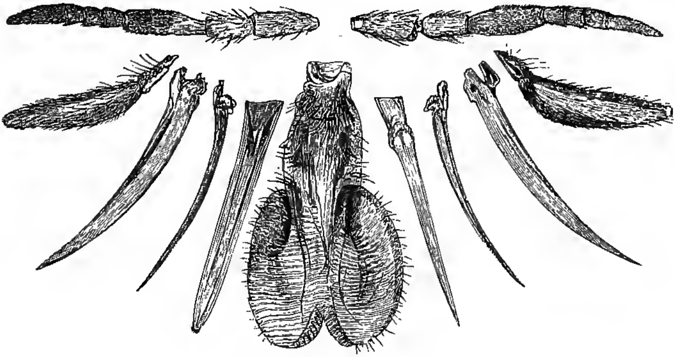

FIG. 142.—Trophi of Tabanus

The further development of this type of mouth is well seen in the organs of a *Tabanus* or Breeze-fly, fig. 142. These are here shown laid out separately, with the antennæ added, as a specimen of the mounted 'trophi' already recommended. The general strong resemblance in the several organs is easily seen at a glance, and also that the line of development is carried still further, the fleshy *ligula* or proboscis being diminished, while the offensive organs are increased in power, till they appear like half a dozen different instruments from a cutler's shop.

In Bees the mandibles remain, and both pairs of palpi, but other organs are modified into the tubular proboscis, so well known. In Lepidoptera the mandibles are mere rudiments, and the proboscis is immensely long, and kept when not in use curled up into a spiral. It is really in two halves, the tube being made up of two half-tubes or gutters with their open diameters in contact.

**Antennæ.**—Many of these organs are of exceeding beauty, especially the feathered or plumed ones of many Lepidoptera and of the Gnat family. They differ to an altogether extraordinary extent in different orders, and even different genera. They appear in very many orders to have most to do with the mysterious power the males of many insects possess, of finding the females; so that a crowd of some moths will gather round an unpaired female, even when concealed in a pill-box about one's person. At all events, in a large number of cases, as the common Cockchafer—the antennæ are immensely more developed in the male; and when this is the case it is well to mount both sexes upon one slide.

Eyes have been sufficiently dealt with on p. 202. Such exquisite sections can be obtained professionally mounted, that it is a pity not to avail oneself of results which can hardly be rivalled by any amateur.

**Ovipositors.**—The ovipositors of female insects offer immense variety in the different orders. In Crickets and Locusts they are very large indeed. The most interesting are perhaps those of the Hymenoptera. In Ichneumons they are mostly long and pointed. In some genera they become boring organs, as in the great *Sirex gigas*. In Bees and Wasps they are connected with poison reservoirs and become *stings*, which are accordingly confined to the female sex entirely, the males being helpless. More or less serration at the tips belongs to nearly all. In Bees and Wasps the teeth are *barbs*; but the most interesting development of this toothed feature is in the Saw-flies, of which there are many genera. The saws of a very common fly are shown in fig. 143. In every case the saws are in pairs, and each 'blade' or cutting-saw works backward and forward in a grooved sheath which embraces its back edge. The two saws work side by side with alternate motions, and thus cut a groove in the leaf, or rind, in which the eggs are to be inserted. The exact

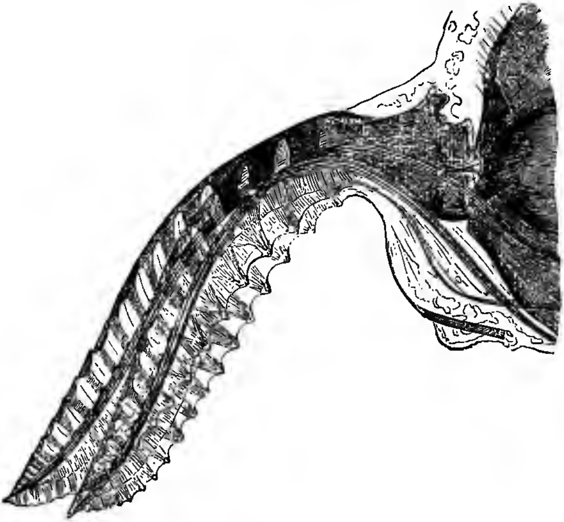

FIG. 143.—Saws of Saw-fly (× 40)

pattern of teeth differs somewhat; but a very curious fact is that most of them, as can be seen from the figure, almost exactly resemble a pattern which has been adopted in some surgical saws, and some saws made in America for various purposes.

**Eggs.**—Ovipositors naturally remind us of the eggs of insects. Many of these are very beautiful objects. Fig. 144 shows some eggs of butterflies magnified, while the upper right-hand figure illustrates the natural size, and the manner in which they are generally deposited. This however differs a great deal; some being attached to a kind of stalk, and others laid in coils or spirals round small twigs. They are best mounted 'dry' upon a black ground.

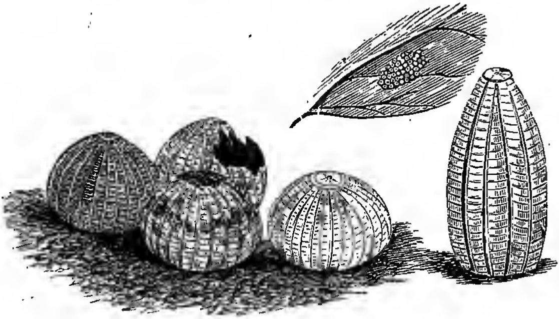

FIG. 144.—Butterfly Eggs

Still more striking are the eggs laid amongst the feathers of birds by the numerous parasitic insects which infest them —each genus of birds, as a rule, having its own parasite, as each genus of animals has its own flea. Fig. 145 reproduces by permission a beautiful drawing by Dr. Dallinger of three of these eggs. That numbered 1 is the egg of a parasite of the Peacock; No. 2 that of a parasite on the Hornbill; and No. 3 is from the Australian Mallee-bird. But analogous forms are to be found in the plumage of our home birds, game, and even of domestic poultry.

**Spiders.**—Various points in these wonderful creatures are worthy of microscopical demonstrations. Their *eyes* are single instead of compound, and usually eight in number, arranged as an upper and lower row of four each. By skilful section-cutters, thin sections of these are prepared, showing the lens, and the rods of the retina. The *head and jaws* are easily mounted, and convey a vivid idea of the terrible offensive *power* of these creatures in proportion to their size. Imagine a cat with a pair of claw-jaws the size of those of a large crab, and with poison in the hollow fangs: that is the scale on which a spider is armed. But perhaps most general interest attaches to the organs associated with the web and its formation.

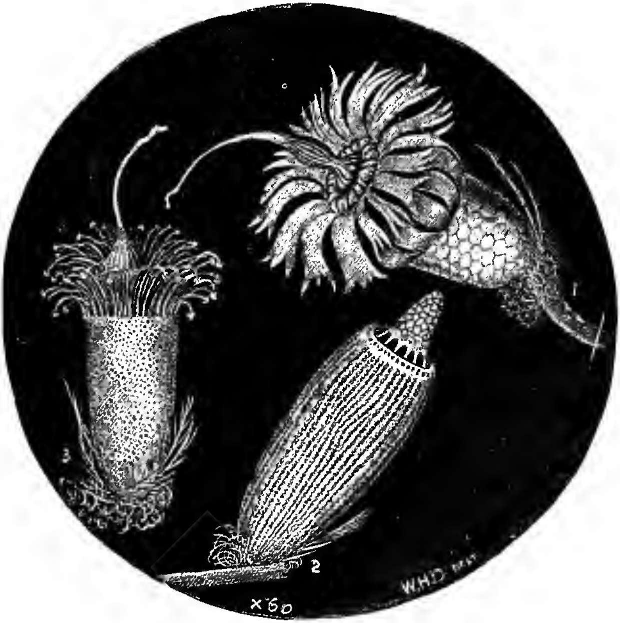

Fig. 145.—Eggs of Bird Parasites

Fig. 146 shows the cloaca and spinnerets of the Garden or Geometrical Spider. The Spinnerets at a first glance appear four, but are soon seen to be six in number. They are not single teats or tubes, but contain in some species up to a thousand separate tubules. The microscope itself will make apparent, that the lower pair contains tubes much larger than the others. But it has required attentive observation to discover, that these large tubes emit a more liquid and viscid secretion than the others, and that the spider has the power of ejecting filaments from the hundreds of pores at once, either so that they remain separate threads, or so as to unite almost immediately into one stronger thread. She spreads them when making the holding end, or anchorage of a line, before it unites into one; also she spreads them when winding up a victim in a shroud of silk, turning it over and over with her legs whilst emitting the tangle of silky filaments. In ordinary web-spinning she converges them, so as to join into one cable almost at once. Statements about her 'hand-spinning' are fables.

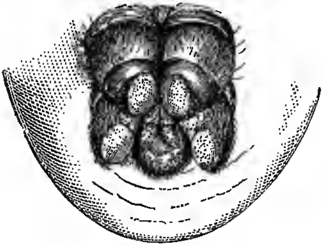

FIG. 146.—Spinnerets

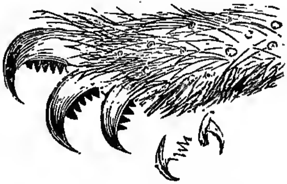

FIG. 147.—Foot of Garden Spider

Yet the foot has to be in relation to the lines, upon which the insect is constantly running with the greatest rapidity. It is terminated by claws armed with many comb-like teeth. The great claws may be two, but in many garden sorts are three, with smaller claws which can oppose them as well—

the latter are shown separate in the figure. However quick the pace, the line falls between some pair of the teeth in the comb, and the foothold is sure and steady.

The radii of the web are spun with ordinary silk (which can in fact be gathered and spun *as* silk, were there enough to be worth while) which is not at all adhesive. By these the spider runs from the centre to her prey. She spins these first, after her marginal foundations; and next spins a continuous Archimedian spiral thread from centre to circumference, of the same non-adhesive thread. She then goes back from the circumference to the centre with another spiral; but this time the *large* spinning tubes are also brought into play, and the hard thread as it is spun is covered with *viscid non-drying fluid*, which appears chiefly as globules strung upon the thread. Larger globules at pretty regular intervals, have generally very tiny ones interspersed all along the intervals, as in fig. 148. It is these globules which detain the flies.[1] They adhere to the spider also, if her foot touches a globule, contrary to the common belief. She constantly has to tear away rungs from her ladders to get free of such mishaps, and as constantly repairs them: and in some cases at least, unless a fresh web is made altogether, which is often done, the adhesive lines have to be renewed next day, the fluid probably drying.

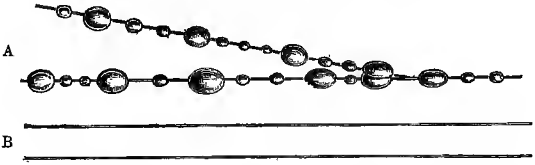

FIG. 148.—Spider Threads A. Adhesive B. Unadhesive

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[1] It is often stated that in spinning the viscid set the spider cuts off and eats the unadhesive spiral, which is used as a bridge only. Without affirming that this never occurs, I am absolutely certain that it was not done in cases which were watched. It may be that species differ in that point.

Much has been written, in ignorance of the true state of the case, about the wonderful skill of the spider, and her dexterity, in forming such a number of these viscid globules so rapidly. If it be remembered that there are hardly ever less than 100,000, and often a quarter of a million, in a web spun in less than an hour, it will be seen that such dexterity is impossible. The truth is far more really wonderful, than any such mere marvel would be (if physically possible). Years ago the late Mr. R. Beck observed that these threads when first spun were quite smooth, but that first undulations and then the globules rapidly appeared. But only quite lately has Professor C. Vernon Boys, F.R.S., shown clearly that if a fine quartz fibre be stroked with a straw dipped in castor-oil, the fluid in a few moments forms undulations and then drops, in just the same way; nay, these drops of castor-oil are equally effective in catching flies. This, then, is what happens. The tough thread, as fast as it is spun, is coated with the fluid; and the web-spider's mode of subsistence, and her complicated spinning organs, are bound up with, and work in association with, those profound molecular laws which cause a jet of water to break into drops, or hold together a soap-bubble!

**Life Histories.**—Only one more line of work can be indicated here. There are many cases, especially where very minute insects are concerned, in which the use of organs, or the complete life-history, is yet unknown. Three illustrations must suffice.

More than thirty years ago (1863) Sir John Lubbock discovered that a minute hymenopterous insect (called by him *Polynema natans*) descended into water, both swimming and crawling therein. Since then the insect is only known to have been observed three times until quite lately (May 4, 1895), when it was rediscovered near London by Mr. W. Burton and Mr. F. Enock, and kept under observation. The first result was the discovery of the unknown fact, that the specimen observed never left the water during several days! Possibly its history may be completed by these observers; but when it is added that some allied species only measure $\frac{1}{75}$th inch long, and that there are other insects quite as small (the *Trichopterigia* among beetles being only $\frac{1}{30}$th to $\frac{1}{50}$th inch) it will be seen what lines of inquiry amongst the more minute insects await the microscopist.

There is another class of histories, in those insects which present the phenomena of alternate generation. Thus, the Aphides will go on for generations producing only females, which bud off as if were, asexually; while late in the autumn true males and females appear, which lay eggs in some sheltered place, thereby preserving the species through the winter till next spring. But M. Lichtenstein discovered that some Aphides presented more complex phenomena, *several* different forms appearing in succession before the final true males and females appeared. It has not been determined yet how far this complicated system of generations is common to the family; but it is pretty certain that there are many Aphides whose full history is unknown.

Our last illustration is from the Gall Flies, and that member of the *Cynips* family which produces oak-apples will do as well as any. Everybody knows the oak-apple, which is a large gall formed by irritation of the tissues in the *twigs* of oak-trees, by maggots hatched from eggs laid therein by flies of the Hymenopterous order. On the gall the maggot feeds, changes to a pupa, and hatches into a hymenopterous fly. All this seems like the ordinary development of an insect. But it is only half the story. The flies thus developed do not deposit eggs on the twigs again, but on the *roots*, where each gall is only as large as a pea; many galls being however often fused into one mass. The galls are also this time *reddish*, and not golden-brown as the oak-apples were. These galls are formed in autumn, and the larvæ hatch in winter, and are pretty similar to the others. But the perfect insects are so widely different, that till quite recently they were classed in different *genera*. They are all without wings, and all females only, which lay their eggs in embryo buds on the twigs; these eggs forming oak-apples in turn. These facts were discovered by Dr. Adler; and since then many other double forms of galls and insects have been discovered, in a few of which the second form develops *both* sexes, though of different forms to the first. In nearly all these cases the two forms were formerly described as distinct genera, and the eggs are laid and galls formed in different situations, and are of totally different appearance.

There is little doubt that patience and research will discover other cases of these double forms, and perhaps associate into one descent other insects now considered distinct. But we have said enough to indicate more than one line of work calculated to afford the greatest interest, in the wonderful Insect World.

## CHAPTER X

### THE VEGETABLE WORLD

UNDER this heading, again, we can only briefly indicate some directions in which the microscopist may find interesting and profitable occupation.

**Cells.**—Thin slices of the cuticle of leaves, or of seeds, and sections in various directions of any tissues, or *hairs* from any plant, removed and placed on a slide under a cover-glass with a drop of water, will soon give a general idea of the numerous *cells* of which any highly-organised plant is composed, and of their great variety in form and character. The tissues of the whole plant are at once seen, far more easily than in the case of animals, to be built up of many single cells.

**Single-celled Plants.**—If the student possesses already a little skill, and a really excellent $\frac{1}{6}$th which will bear a high-power ocular, a small particle of yeast will enable him to study cell-growth and multiplication in a very simple form: but this plant is too minute and transparent for beginners.

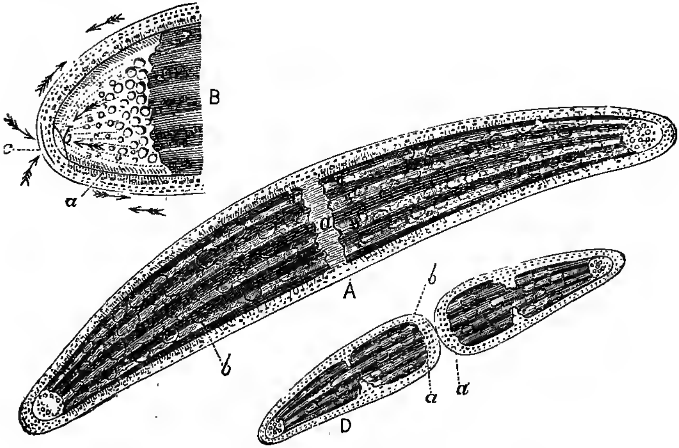


Fig. 149.—Closterium lunula

A. Perfect cell B. One end enlarged to show cyclosis D. Cell just divided

*Bacteria* are not treated here for the same reason. The serious student will have recourse to one of the many special treatises, and will require a good $\frac{1}{6}$ and an immersion. The ordinary ones can be well seen with a *good* $\frac{1}{8}$ or $\frac{1}{6}$ and compensating ocular, and slides can always be procured at 2*s*. or 3*s*. each.

*Desmids* grow in quantities in most ponds, especially those in which the water *looks green*; that colour being usually given by swarms of these plants, on which many aquatic creatures feed. Some may be got in the net: others require to be scraped off the bottom, or from the stems of submerged plants. They are of great variety in form, some of which are very beautiful: we can take but one example, from the group of *Closteria* (fig. 149). This is a very common Desmid, and shows clearly the main phenomena of the family.

First of all, it is easily seen in this Desmid that the living cell is full of living protoplasm, whose constant motion in streams presents the phenomena of *cyclosis* (p. 238). The stream of particles is most clearly seen along the edges, but with care can be made out anywhere under the enclosing membrane. Every now and then it carries along with it one of the larger particles (marked *b* in A) into the chamber at the end. These larger particles are not found in the central dividing space (*a*) between the two halves of the Desmid; which leads to the inference that the cell in this state (A) is already partially divided into two. At B the end is shown much more enlarged. It is then seen that the inner membrane or endochrome is furnished with *cilia* (B, *a*) which are in motion. The protoplasm outside the endochrome shows currents in opposite directions, as denoted by the arrows: in the end chamber are also very strong currents shown by the arrows converging towards *b*, and these currents appear thrown back or reflected as by the arrows pointing to *c*. The whole gives a very vivid idea of the active *life* going on in the cell.

At D is shown the simplest form of propagation, by cell-division. The endochrome retreats more and more from the central space *a* in diagram A, while there is outer constriction at the same time, till the two separate entirely as at *a a* in diagram D, the exterior becoming rounded as at *b*. The exterior ends *b* then elongate, while each half begins to constrict in the middle; and in about six hours each half has become symmetrical or nearly so, like the parent.

This and other Desmids present also at intervals the phenomenon of *conjugation*; two individuals uniting,

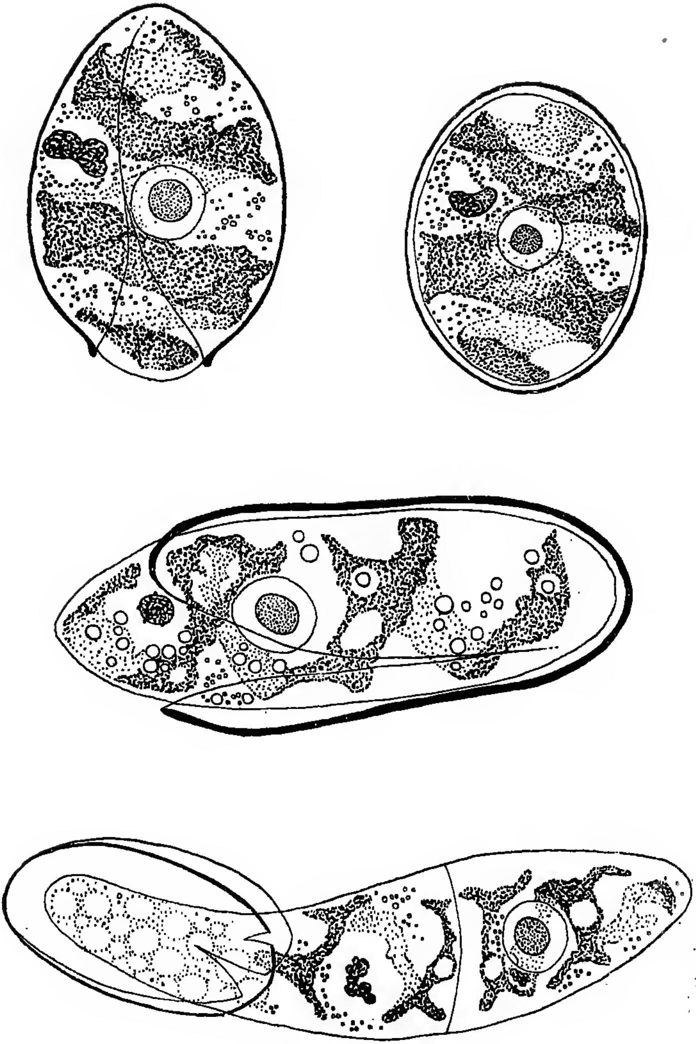


FIG. 150*a*.—Germination of Spores of Spirogyra

and producing a *zygospore* which forms a fresh series. Some good fortune as well as patience will be required to secure specimens in the stage of conjugation. This simple conjugation of two cells apparently precisely alike (though it cannot be proved that it is really indifferent what cells enter into union; there may be differences, of the nature of sex, which the microscope cannot discover) is evidently the earliest steps towards the truly sexual mode of propagation.

**Algæ.**—Almost every pond open to the sun will furnish a variety of these plants, mostly in the shape of long green filaments, which only need to be placed upon a slip with a drop of water and a cover-glass. In these filamentous forms we have a number of similar cells joined together end to end, the simplest form of a complex structure.

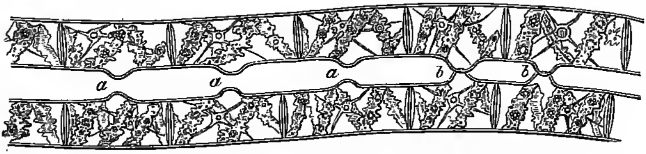


FIG. 150.—Spirogyra conjugating (*Sachs*)

Many of the plants are of great beauty when seen on the stage, especially those of the *Spirogyra* family. The cells of this group for a while divide in the middle, each half lengthening so as to extend the filament; and during this period the endochrome and chlorophyll bodies usually remain a compact mass. But as the cells mature, the endochrome contracts, and shapes itself into a beautiful *spiral* green band inside the outer tube, from which the family takes its name.

The *Spirogyra* furnishes another example of conjugation between two cells similar to all appearance: but there is a distinct further advance in the process. Two adjacent filaments whose cells are mature, throw out from each cell lateral protuberances (fig. 150, *a*). These grow till they meet (*b*), and meantime the spiral contents of each cell shrink and gather into an ellipsoidal mass. Then the cell wall opens between the two projecting parts, and one of the two ellipsoidal masses passes gradually through to coalesce with the other. Close observation, however, shows that the spiral band is preserved, though forced together into a compact body; and moreover after coalescence the two bands are so arranged as to form one band. The passage of one mass into the other is shown nearly complete at *a* in fig. 151, and quite so at *b*. The 'zygospore' thus formed remains apparently quiescent for some time, often months; and then begins to elongate and divide to form a new filament.

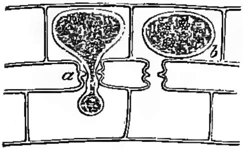


FIG. 151.
Conjugation complete (*Sachs*)

It is to be remembered that, while the two cells show no observable difference, the contents of one are *entirely* absorbed by the other: also that all the cells in one filament, are usually absorbed by the cells in the other. These facts appear to indicate that there *is a difference* in the two cells, though it eludes present observation; and that we have here a further advance towards difference and union of sexes.

**Alternation of Generations.**—We further have in the above an early stage of the phenomena called Alternation of Generations. Just as in the Aphides (p. 221) we had generations budding from imperfect females only, to be succeeded at intervals by true union of sexes; so here in the vegetable world, we have multiplication for a period by division and growth of cells only, but followed after a while by union of two individuals. These so far appear alike; and yet we have indications that already they are in some way different. But in some form or other, Alternation of Generations between some form of cell-multiplication, and the formation of new individuals by conjugation of two, prevails through the entire vegetable world. The details of Fern propagation are very remarkable, but must be studied in botanical works: in this class of plants alternation is regular. Potatoes have been propagated almost entirely for generations by 'eyes' or tuber-buds. In mosses, it is the asexual product usually observed. The *Chara* family form at intervals perfect fruits; but there is one species—*C. crinita*—in which only female organs have been found for many generations in most Continental localities, though a very few male organs have now and then been observed in Transylvania. There are many various phenomena of this kind, which are not fully understood, and will furnish subjects of profoundly interesting microscopic research to the botanical student.

**A Life History.**—As just one illustration, let us take the history of the common mildew or rust in wheat, which has only lately been established by the researches of De Bary, and which may be compared with that of the Gall Fly (p. 221). As in that case, investigation has shown that what were once considered fungi of different genera, are but stages in the history of one. There are found in spring on the leaves of the common Barberry orange-coloured swollen spots, which under a powerful hand-magnifier present the appearance of clusters of small cups, as in fig. 152. On making vertical sections, the swollen parts are found to be full of the thread-like *mycelium* of a Fungus. There appear however to be two forms of fruits, which themselves were once considered two fungi, but are now known to be but two forms of one, as shown in fig. 153. Here $x$ is the natural thickness of the leaf, which is so swollen by the thread-like mycelium. From the mass protrude on one side the 'spermogonia' *sp*, urn-like receptacles lined with mycelium, whose threads protrude through the epidermis. From these receptacles are detached minute bodies called *spermatia*.

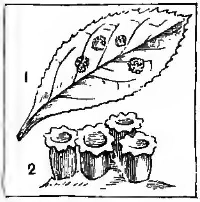


FIG. 152.—Cluster Cups
1. Nat. size
2. Magnified

The other form of fruit is much larger, and is the old *Æcidium* of botanists; a name still used, but only as a stage in the history of the mildew *Puccinia graminis.*

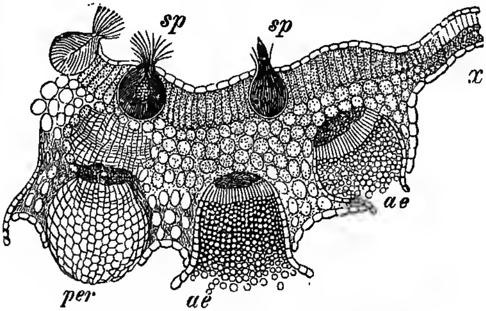


FIG. 153.—Section of Barberry Leaf (*Sachs*)

These also spring from the mass of mycelium, and at first are nearly globular bodies *per* beneath the cuticle, lined with a layer of cells called the peridium. These finally break through to form the open æcidium cups *ae,* and which give off chains of larger æcidium spores. As will be seen, a section showing these stages of development makes a very beautiful microscopic object.

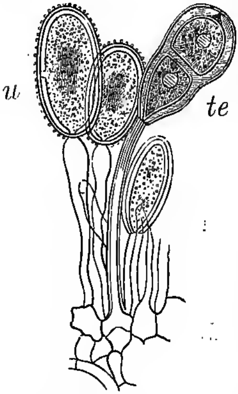


FIG. 154.
Uredo-spores (*De Bary*)

These spores (whose union in some way with the *spermatia* is probable, but has not even yet been proved) cannot germinate upon the Barberry. But on the stem or leaf of wheat or grass, they throw out germinating filaments which enter the pores or stomata and form another network of mycelium there. This mycelium produces another form of spore or fruit known as *uredo-spores* or fruits, and shown at *u* in fig. 154, which are borne at the ends of filaments, and are known as the 'mildew' on the wheat. These uredo-spores germinate on the same grass or wheat, and the mycelium from them forms similar spores, this going on for several generations. At last the *older* uredo-plants begin to produce amongst the others, at first single specimens *te* (fig. 154) of another and larger two-celled kind of spore, known as *teleutospores.* Then the uredo-spores cease, and the uredo-plant produces *only* groups of teleutospores, as in fig. 155, which break through the cuticle of the wheat-plants. These teleutospores remain on the haulms through the winter, and in the spring they germinate and throw out filaments, at the ends of which yet another kind of sporidia are produced. These germinate and produce mycelium only when they find a lodgment on the surface of the leaves of the Barberry, with which event the cycle commences over again.

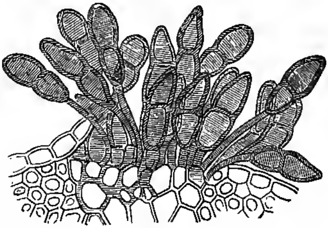


FIG. 155.—Teleutospores (*De Bary*)

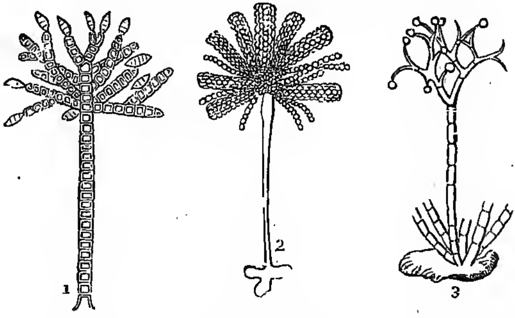


FIG. 156.—Moulds

**Fungi, Mosses, &c.**—All the lower kinds of plants present beautiful objects for the microscope, but cannot be particularised. Almost any kind of 'mould' becomes, when magnified with a low power, a miniature forest of great beauty. Higher power reveals the cell arrangement. In fig. 156, No. 1 is a mould growing on the stem of a plant; No. 2 *Aspergillus glaucus*, the cheese-mould: No. 3 a mould found on many decayed vegetable substances.

Many of the Mosses, Lichens, and Liver-worts are also interesting objects. We may just mention the *peristome* of many Mosses. The product of fertilisation in these plants is a *theca* or urn, which is at first closed by a lid. When the fruit is mature this lid falls off, and the mouth of the urn is then found surrounded by a toothed or fringed structure—the *peristome*. Fig. 157 shows a very beautiful peristome in which the fringe is double, the inner one being joined by cross-bars so as to make a kind of trellis-work. There are many beautiful varieties of double as well as single peristomes to be found in the Mosses, and a representative series would make a beautiful and interesting collection of objects.

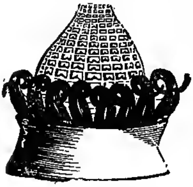


Fig. 157.
Double Peristome
(*Fontinalis*)

**Development of Sex.**—We can hardly help seeing, even from the few facts mentioned in p. 224, how very deep down and fundamental is the phenomenon of *sex* in the great realm of organised Nature. Even in the kingdom of plants, only in the very lowest types are two conjugating individuals alike, or can one take the place of the other; if indeed it is possible even in them, which we have seen reasons for doubting. The higher we get in organisation, the more obviously *different* in appearance, as well as work, do the two beings become, and the more utterly impossible for one to fulfil the functions of the other.

This development may be traced very low down. There is a filamentous Alga called *Sphæroplea* which is composed, like *Spirogyra*, of many single cells joined end to end, and which multiplies by lengthening and division for a time. But when mature, instead of two (apparently) similar cells conjugating as in figs. 150, 151, some cells develop into sporanges, while others are filled with small active bodies with flagella, called antherozoids. A small aperture forms in the side of each kind of cell, by which the antherozoids escape from their own mother-cell, and enter the other cells, which are thus fertilised.

As we go higher the organs become more complex. There is a small fucoid plant common in marshes, called *Vaucheria*, which at one stage expels from the ends of its club-shaped filaments large spores, remarkable for being completely *ciliated* all round their exterior: these are considered non-sexual zoospores. When the mature stage has arrived, the filament develops two *different* organs very close together, short and broad but rather curled, as in figs. 158, 159. The *sporange* A is an enclosed vessel filled with cells; the adjacent *antheridium* B (fig. 158) is filled with active antherozoids. The sporange bursts open first, and the antheridium soon after, when the antherozoids enter the sporange and fertilisation takes place, the result being a kind of fruit-spore as in fig. 151, containing several brown bodies. This large spore it is which sprouts into the next generation, after an interval, usually of three or four months.

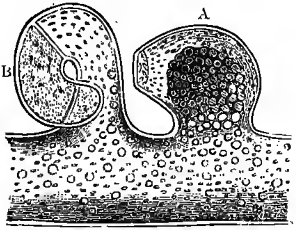


FIG. 158.—Vaucheria Before Fertilisation

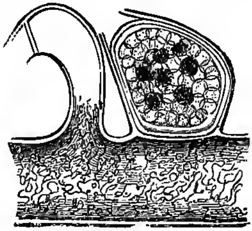


FIG. 159.—Vaucheria After Fertilisation

In the Mosses and Ferns the analogous organs are further developed, into pairs called *antheridia* and *archegonia*, many of which form beautiful objects for the microscope, especially when cut into very thin sections.

**Flowers and Fertilisation.**—It is in the true flower-bearing plants, however, that we find reproductive organs most perfectly developed. In these the antheridia become *anthers*, carried on the ends of stamens, and forming *pollen-grains* instead of spermatozoids. These pollen-grains are of very various forms, a few being shown in fig. 160. They are easily collected, and many make beautiful slides mounted simply in benzol-balsam. Sections across the anthers also make fine objects if skilfully made, and will show the pollen-grains within.

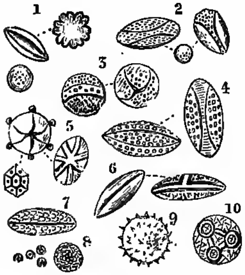


Fig. 160.—Pollen-grains
1. Primrose 2. Auricula
3. Anemone 4. Iris
5. Clematis 6. Tulip
7. Rose 8. Buttercup
9. Hollyhock 10. Passion Flower

The female portion of the flower comprises the receptacle called the ovary, containing from one to many bodies called *ovules*, each of which (if fertilised) becomes a seed. From the top of the ovary extends a column termed the *style*, composed of soft tissue, the *stigma* or extremity of which exudes when mature a viscid and sticky secretion, easily seen on any fair-sized stigma with a pocket magnifier. The unfertilised stigma presents no opening whatever.

If pollen-grains are placed in water, they often burst and disperse numerous particles; and it was formerly thought that these were a kind of antherozoids, and were dispersed upon the stigma, to work their way to the ovules. Not until 1822 did Amici discover that this was not so, but that the pollen-grain, on adherence to the viscid fluid, began to push out and *grow*, first as a short protuberance; then a short tube; until this tube gradually grew all the way down between the cells of the stigma. This is represented in fig. 161, showing a section of the stigma of a Thorn-apple. The stigma with its viscid surface is shown studded over with pollen-grains, which have grown downwards as shown by the dark shading. To exhibit this in section will tax the skill of the microscopist, especially in section-cutting. The stigma of the *Œnothera* or Evening Primrose is one of the easiest to manage; and the best way is for the operator to artificially apply pollen to mature stigmas, and then to section one morning and evening every day, till he gets what he requires. A single tube can rarely appear in the section for any great length; but the most interesting portion is that near the original pollen-grain.

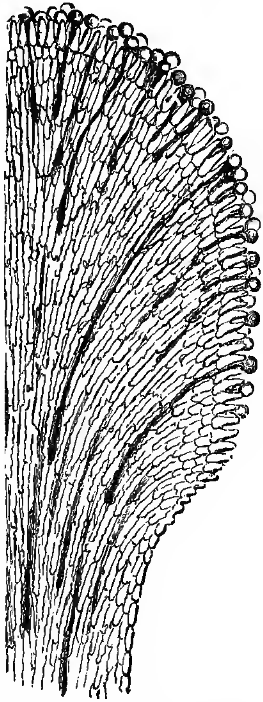


FIG. 161.—Pollen-tubes growing

This growth of the pollen-tube is a most wonderful phenomenon. Its length is often hundreds of times the diameter of the pollen-grain, and may be as much as two or three inches in some plants. The question suggests itself as to how it occurs. The answer is not doubtful. The viscid sugary secretion first nourishes the pollen into growth, but the tube quickly grows beyond this source of nourishment, opening for itself a kind of canal down the style. The cells lining this canal become however *also charged with sugary matter*, which continue to nourish the pollen-tube, and further stimulate its growth. This secretion of nutritive matter is plainly produced by the *irritation* caused by the pollen-tube, as it pushes its way farther and farther down, the phenomena being analogous to the secretion caused by Gall Insects, described in p. 220. The lining of extra-nutritive conducting-tissue around or touching the pollen-tubes, can only be perceived with good lenses, in thin sections cut *across* the fertilised stigma.

At last the tube or tubes reach the bottom part of the style, pass into the cavity of the ovary, and there enter the minute orifice or micropyle of each individual ovule (if more than one) as seen in fig. 162, which represents the style and single ovule of buckwheat. Thus the germinal vesicle in the embryo-sac is fertilised, and certain further changes commence, which change the ovule into a seed or fruit, embodying for the next generation all the work of the past, all the hope of the future

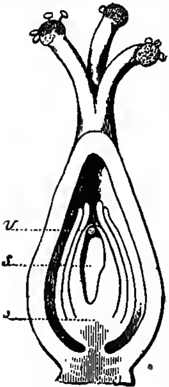


Fig. 162
Style and Ovule
(Buckwheat)
o. Ovule
v. Germinal vesicle
s. Embryo sac

It cannot be here detailed how every thing in Nature is made to bear upon this end, in the greatest variety of ways. It is easy to write volumes upon the 'Loves of the Plants,' and such have been written. The scents and colours of flowers are all to serve this end; the visits of insects, with nectar to tempt them, and with colour and odour to attract them, being relied upon to bring the pollen of one flower to the stigma of another. In not a few cases *one insect alone* is able to do this. In other cases the wind performs the hymeneal office, and appendages to catch the wind are provided. In many cases the pollen fertilises the stigma of its own flower. Anthers and stigma combined in one flower are very common, but it is also common to find the sexes on altogether separate flowers, as in the willow and yew. But all such marvellous instances of adaptation belong to Botany rather than Microscopy.

Sections of flowers, and ovaries, and buds, both transverse and longitudinal, are very beautiful objects. Being soft, they are prepared with no special difficulty.

**Stems.**—A few words must suffice for the structures found in plants. Of the preparation of sections of Stems, in general, and their staining or double staining, sufficient for the purposes of this book has been said in pp. 139–142. Shoots or branches from $\frac{1}{4}$ to $\frac{1}{2}$ inch diameter are most suitable; but this must depend upon the rate of growth. If possible, three sections should be prepared of each—one transverse, one longitudinal in a radial direction from the centre of the shoot, and a third also longitudinal, but cut at a tangent to that centre. In regard to exogens, or those plants which add rings every year, it is a good plan to prepare three transverse sections, of one, two, and three years' growth. The common pine shows the annual rings clearly.

Endogens, on the contrary, exhibit independent bundles of fibro-vascular tissue over the area of the stem. Many of them, well cut and stained, are of exquisite beauty. Roots also offer many interesting sections.

**Vessels.**—The stems and other main parts of plants mostly consist of longitudinal vessels, which are formed essentially by the elongation of cells and thickening of the cell walls. We cannot go into detail; but fig. 163 shows one very characteristic type, in which the harder thickening material is arranged in spiral form, just as the chitine in the tracheal tube of an insect (fig. 124) shown for comparison in the same figure. Spiral vessels are easily, after a little maceration, teased out of a piece of the stem of the common rhubarb, and of many other plants, especially from the larger veins of soft leaves.

If the rings are separate, and more or less complete, we have *annular* vessels, or the thickened walls may be dotted over with thinner spots, which give punctated vessels. In some plants, Ferns especially, the walls of many vessels are more or less polygonal, and the rings very close, and connected by very short longitudinal fibres: these forms are known as *scalariform* vessels.

In woody fibre the cell-walls are much thickened and hardened. The most interesting form of woody tissue is found in deal and other *coniferous* woods. Here each long cell is studded with thin spots surrounded by concave depressions, on the flat faces which are turned towards the medullary rays, or radial planes from the centre of the stem. The flat face of the cell with the pits is shown at A, fig. 164, and the lenticular cavity formed by two pits (which always face each other) is shown in section at B. A very thin slice from a lucifer match will show these pits; but to get the two positions perfectly, sections must be cut both radially and tangentially as regards the stem.

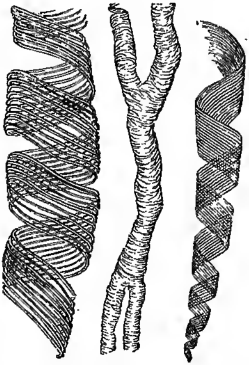


FIG. 163.—Spiral vessels (Trachea of an insect shown in centre for comparison)

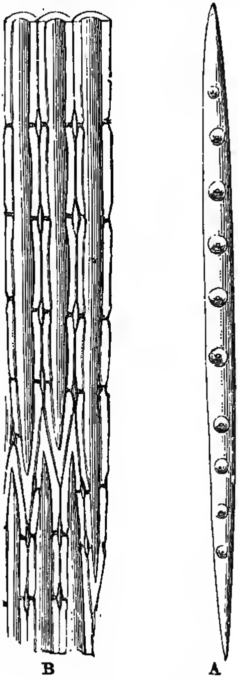
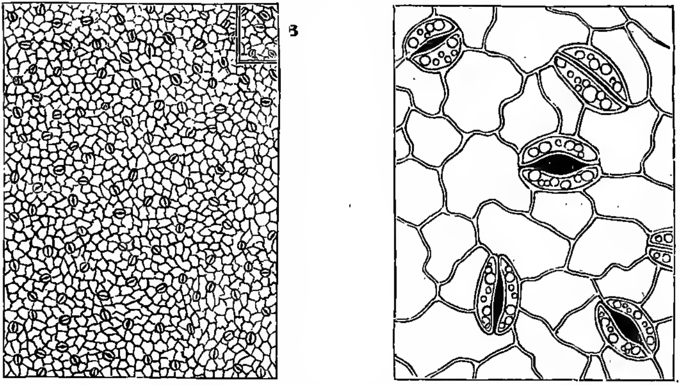


FIG. 164.—Pitted vessels

**Leaves.**—These offer many interesting objects. Separating and staining a piece of the under cuticle, or surface layer of cells, this will in most cases show under a quite low power the *Stomata* or apertures, leading into cavities in the cellular tissue, by which plants 'breathe' (fig. 165, A). With a higher power it will be seen (C, which shows the little piece marked B in A, further magnified) how each aperture is bounded by two kidney-shaped 'guard-cells.' The cuticle will also exhibit cellular structure plainly.



A. × 100 C. × 700

FIG. 165.—Stomata of Lilac Leaf

Vertical sections of leaves should also be prepared, and are very interesting. Under the cuticular layer, is usually one of deeper and narrower cells, often termed 'palisade' cells (fig. 166). Beneath this the cellular arrangement varies much, but as a rule the upper layers are dense, whilst amongst the lower layers are many cavities, into which the *stomata* open. A great deal depends on chance; but out of a few sections one or two should exhibit some of the *stomata* in section, as in the figure. Some of the thicker leathery leaves, such as laurel, or of the India-rubber plant (*Ficus elastica*) make fine vertical sections with the least trouble.

Portions of the fronds of Ferns may be mounted either dry, or transparent. Many petals of flowers, which are forms of leaves, are lovely objects. A favourite one is a petal of geranium, stripped of its cuticle, then dried, immersed in turpentine for two hours, and mounted in balsam. The petal from a flower of the common Chickweed is another beautiful object, and shows in the 'veins' spiral vessels in their natural position.

The cuticles of many leaves are studded with *hairs* of various shapes. Many cells of leaves and other parts contain *crystals*, either singly or in masses. In others there are grains of *starch*, which phenomenon is best shown in a thin section of potato. Hard tissues such as the 'stones' of fruits; thin cuticles such as enclose the edible part of a walnut; pith; rind; all these and other structures present various forms of tissue built up of cells, for further details of which reference must be made to works on Structural Botany or the Physiology of Plants.

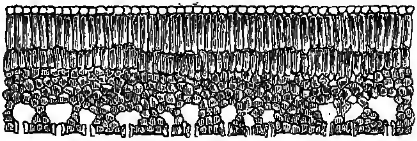


FIG. 166.—Vertical Section of Lilac Leaf × 100

**Cyclosis.**—We must conclude this chapter by reference to one phenomenon of living cells, which more than any others impresses the observer with the idea of vital activity. This is the circulation in each cell of the living protoplasm, in a current round the outer walls, which can be best traced by the current carrying with it green particles of chlorophyll. The probability is that all cells containing fluid protoplasm present more or less of this motion (which must not be confounded with the *circulation* of sap through the whole plant: it is simply a current round the interior of *each cell*). But only thin cells with transparent walls are suitable for observing the phenomena.

The hair of a Nettle, or still better of the *Tradescantia*

*Virginia,* detached and put on a slip with a drop of warm water, shows this phenomenon well. So do the terminal shoots of the aquatic plant *Chara,* or the thinner ones of the allied *Nitella.* A leaf of *Anacharis* treated in the same way shows this protoplasmic movement (termed 'cyclosis') well, and with no trouble, in the cells up the midrib, or round the edge of the leaf. But it is best shown, though with rather more trouble, in a piece of leaf of *Vallisneria.* A thin slice should be shaved (with a lancet is easiest) from the surface of the leaf, so as to come as near as possible to a single layer of the cells, and put on a slip with warm water. Such a thin section is nearly transparent, and the cells are on a larger scale and more regular in form. A fair quarter-inch with a moderate eye-piece, or a high-class half-inch or four-tenths with a high-power ocular, will exhibit the motion of the chlorophyll granules well, as in fig. 159, which is drawn under a half-inch apochromatic.

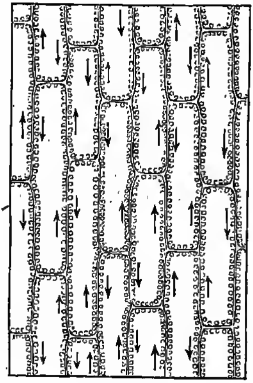


FIG. 167.
Cyclosis in Vallisneria

Generally the detachment of the piece of plant, or of the hair or leaf, as may be used, appears at first to paralyse the motion, as if the cells were really killed. This is however only temporary, and if the leaves or small slices are placed for an hour or two in a small tube full of warm water, or carried in a pocket next the body so as to be gently warmed, the cells are revived, and the motion recommences.

# CHAPTER XI

## VERTEBRATE PHYSIOLOGY

THAT study of the most minute structure of the human or other body which is termed Histology, does not belong to the purposes of this work. Medical or biological students will work out such studies by the aid of the treatises of Schäfer or Klein, and such material as is provided in class. So far as the human body is concerned, material for personal section-work or dissection will rarely come in the ordinary reader's way; and it must suffice to indicate in this chapter a few typical organs or structures of which the microscopist will probably desire to possess a few purchased slides, as being of popular interest.[1]

**Blood.**—The examination of a drop of blood is an exception, being so easily made. Winding a few threads round a finger below the nail, a prick near the side of the nail with a fine needle is hardly felt, and is followed by the desired drop, which can be transferred to a slip. If the cover be placed *on* the drop so as to form a thick layer, the red corpuscles show a great tendency to gather into rolls adhering by their flat sides like a pile of shillings (fig. 168, A). But if the cover-glass is laid on the dry slip and pushed along till the blood is drawn under by capillarity, a thin layer is secured, when the individual corpuscles stand out separately, c. The little disks, averaging $\frac{1}{3500}$ inch in diameter, have rounded edges, and are rather hollowed on the sides. This concave lenticular form results in a difference of focus showing either light or dark centres.

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[1] For those interested in Histology as such, an exception may be made to the recommendation on p. 136 not to purchase 'sets' of slides. From any of the metropolitan microscope houses a set of six dozen slides, mounted in balsam, in a pine cabinet like fig. 70, and representing (according to a reference-list) nearly all the important organs and tissues of the human body, can be obtained for *two guineas*. They are known in the trade as 'Jones' sets,' and are largely used by medical students and teachers. The sections are admirably prepared.

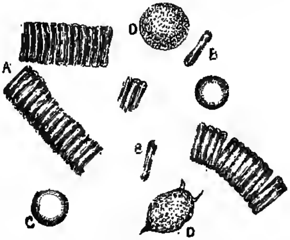

FIG. 168.—Blood Corpuscles
A. Roll of red corpuscles
BB. Red corpuscles in profile
C. Red corpuscle flat
DD. White corpuscles

The corpuscles of the blood of fishes, reptiles, and birds are larger, oval in form, and with a very distinct nucleus. Those of some reptiles are very large in proportion, and slides are procurable of any dealer showing the nuclei stained green, with the rest of the corpuscle stained red.

Here and there appear among the round disks, rather larger corpuscles D D, not so smooth at the edge, and at times with distinct protuberances. They rarely average more than 1 in 250 to 400 of the red ones. If the slip can be kept warmed to blood heat, they are found to change their form and place as in fig. 169. In short, except that they are much smaller than the ordinary *amœba* of fig. 86, they appear to behave exactly the same in every respect. They are, in fact, *distinct living bodies* of the same kind, *taking food* also in exactly the same way. Especially have they been observed to feed upon such *bacteria* as infest the body; and when irritation or inflammation is set up anywhere, by the attack of these disease-producing germs, the white

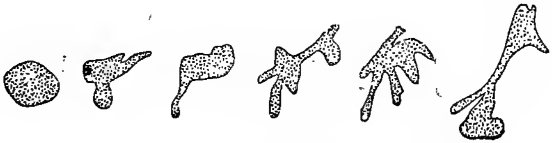

FIG. 169.—Amœboid Changes in White Corpuscles

corpuscles or leucocytes rush to the spot from distant points, and appear to attempt to devour them. The point is still debated; but a large school of biologists and medical men believe that resistance to such attacks of disease-germs, or recovery from the attacks, depends upon whether the white corpuscles of the blood can succeed in devouring the germs before fatal mischief is done.

**Circulation of the Blood.**—The display of this is always interesting. It is usually shown in the transparent web of the foot of a frog; but the tail of a tadpole, or tail or fin of a minnow, are also used as convenient. We will take the frog. The orthodox manner of treating him was to have a 'frog-plate' of brass *a a a* (fig. 170) with holes *g g g* round one end, and an aperture across which the foot is to be stretched. The frog is put head first into a bag *b b*, whose neck can be drawn round the one leg allowed to protrude by the strings *d d*, and by tapes *c c* sewn to the bag, it can be tied securely round the plate, or what is better, to studs projecting from it. A few pins *h* with slots *i* cut in them are provided to fit the holes *g g*. To the principal toes *f f f* threads are tied: then these threads are stretched out, stretching the web with them, and the threads being turned

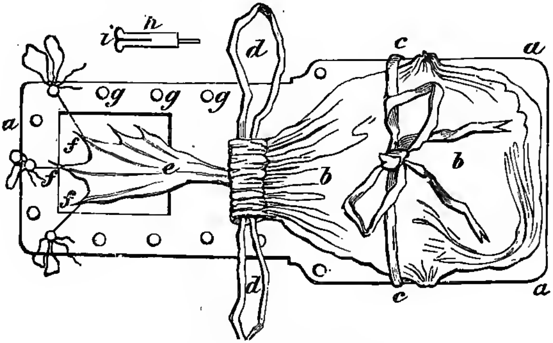

Fig. 170.—Frog on Plate

two or three times round the pegs and then pulled down through the slit *i* in each, the whole is fastened. The bag should be of linen or cotton, not waterproof, and be wetted, or a little wet moss put in with the animal; and the web of the foot also needs wetting occasionally. If the bag or the tapes be tied too tightly, the circulation will sometimes stop, and it should be loosened a little. Mere fright sometimes seems to paralyse the animal; but there is very seldom any difficulty.

But most demonstrators prefer Dr. Carpenter's plan of using a plate of *cork*, with a hole half an inch diameter in the centre. The threads attached to the toes have little loops made in their ends, or are tied round common pins; and these pins are stuck into the cork so as to stretch the web, as with the plate. An inch power is amply sufficient.

**Capillary System.**—The circulation thus seen is near the smaller extremities of the veins and arteries, where the two pass into each other through the capillary tubes. With a little adjustment the capillary connections themselves can be brought into view, the diameter of these tiny tubes being in many cases only sufficient for the free passage of one corpuscle at a time. To exhibit these capillary blood-vessels, sections are prepared of tissues in which the blood-vessels have been *injected* with carmine, so as to show up in bright crimson. Fig. 171 represents a small portion magnified of the capillary system in the lung.

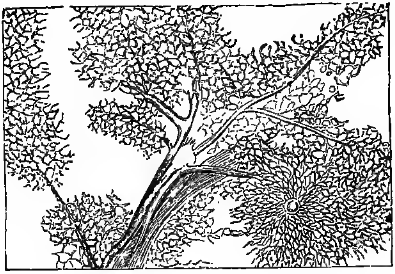

FIG. 171.—Capillary Vessels in Lung

**Ciliated Cells.**—The amœboid white blood-corpuscles are not the only case in which we find grouped, in the higher animals, individual cells reproducing the lower forms of life. It is true that the cellular construction of a vertebrate's body is not (to a very low power) so conspicuous as in the case of plants, in which it cannot be overlooked; but the use of moderately high powers speedily reveals that here also we have a complex life, embracing in it the individual life of innumerable single cells. We cannot enumerate all the various kinds; but one of the most interesting (from this point of view) is the *ciliated* cell.

The ciliated animalculæ were briefly described in Chapter VIII, and the part the minute cilia played in the motion of the animal, or (by causing currents in the surrounding fluid) procuring food or assisting respiration, was alluded to. Taking now an example far above an Infusorian, yet far below any vertebrate animal in structure, if we examine a section through the gills of a mussel, with a high power, we shall see (fig. 172) that the exterior of the gill-leaves or plates is composed of longish cells in close contact, each with a nucleus, and with their outer ends furnished with cilia *c*. These are in incessant motion, and by this motion the fresh water is swept into the gills, and the breathed water expelled. This office is what we should probably expect. The motions of the cilia average some ten or twelve per second; and that the individual *cell-life* is apart from the higher or organic life, is proved by the fact that the cilia are seen to move, hours after a piece of gill has been detached from the animal.[1]

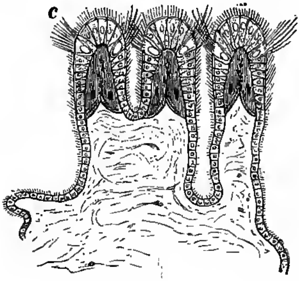

FIG. 172.—Gills of Mussel c. Cilia

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[1] Dr. Carpenter states that the cilia have been seen in motion on the trachea of a criminal seven days after decapitation. And detached ciliated cells will swim freely about in warm water like an Infusorian.

But it is wonderfully interesting to know that we find similar ciliated cells in the highest Vertebrates, including ourselves, and that they perform most important functions. In this case they line *interior* parts of the body, which arises from the mode of embryonic development. If the germ of a hatching egg be kept under examination, the first trace of the future 'plan' of the animal is an open groove down the centre. This groove gradually grows deeper, and at last closes in, into a tube. Thus the originally *outside* layer of cells, becomes an *inside* lining. The internal organs are developed from the cavity thus formed. It is only on some of these surfaces, which are *kept moist* by thus becoming internal surfaces, that the cilia survive. Fig. 173 illustrates a few forms, A being from the lining membrane of the nose, B from the trachea of a cat, and C from the mouth of a frog. They belong to the delicate lining known as *epithelium*. Every cell is seen to be distinct, and to have its own nucleus; but the cells differ in shape. When oblong and regular as in A, they are known as 'columnar' epithelium, and when in addition ciliated, as here, 'ciliated' epithelium. Only a most skilled section-cutter can furnish sections showing these cells; but if the reader has a *good* $\frac{1}{4}$ or $\frac{1}{6}$, he can probably procure detached ciliated cells by scraping the inside of a frog's cheek with a spoon,[1] and placing a drop of the mucus upon a slip with a cover.

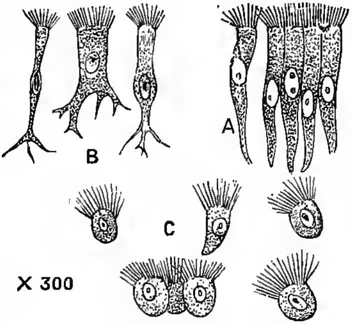

FIG. 173.—Ciliated Vertebrate Cells

The great interest of these cells lies in their position and the work they do. The main positions they occupy are along the spinal cord or marrow, a narrow tube in which is lined with cilia; the cavities of the brain; the windpipe, trachea, and bronchi, and nasal tubes—i.e. the respiratory passages; and the ear passages. How these originally outside appendages came to be on *inside* places, we have seen.

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[1] No more pain is caused than in scraping one's own. This will also produce epithelial cells; but in man these are not ciliated.

Their office is a cleansing and freshening office. In the case of the respiratory passages, it has been ascertained that the cilia keep the fluids lining those passages in continuous motion *towards the mouth*, thus getting rid of dust, or effete and diseased matter. It is when the offending matter gets too much for this action, or when the action of the cilia has not sufficient vigour, that we get distressed, and have to *cough* the matter up. The lining of the nervous cavities probably has to be cleansed in the same way.

**Muscle.**—Of the more obvious structures of the vertebrate body, the first to occur to mind is muscle—the substance of 'lean' flesh. This is of two great classes, sharply distinguished. In all cases where the motion produced by the muscle is *voluntary*, the fibres are striped or striated as in fig. 174. The coarseness, and some characteristics of the striation, may differ in different classes of animals, as in mammals, reptiles, fish, and insects; but striation is common to all. On the other hand, where the motion is not voluntary, as in the intestines, the fibres are plain or unstriped.

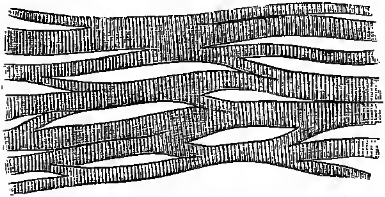
FIG. 174.—Voluntary or Striped Muscular Fibre

Muscle can be prepared easily. A bit of either raw or *boiled* flesh may be teased out with two fine needles into the finest possible fibrils, under an inch power of the single or dissecting microscope. Raw flesh is sometimes teased out more easily if first macerated in a weak solution of chromic acid. The flesh of the pig or the frog yields good fibres, as do lobsters and crabs. Some insects furnish muscle with coarser striation, the *Dyticus* or great Water-beetle being a favourite. The striations are thought to show more plainly, if the muscle is mounted in glycerine or glycerine jelly: but on the whole balsam is to be preferred.

**Bone.**—Sections of bone are interesting. Fig. 175 is a transverse section of human bone, showing first a series of very small tubes (averaging $\frac{1}{300}$ inch diameter) known as the *Haversian canals*, which are in connection with the great central cavity (when there is one, and like it are filled with marrow), and supplied with blood-vessels. Round these are concentric lamellæ of the organic structure, infiltrated with earthy matter. Between these lamellæ are spaces called lacunæ, shown by the black spider-like spots, from which radiate in all directions a multitude of very fine canals—*caniculi.* The lacunæ are generally about one-third deeper than they appear broad, and the proportion between their apparent length and breadth (the section being sup-

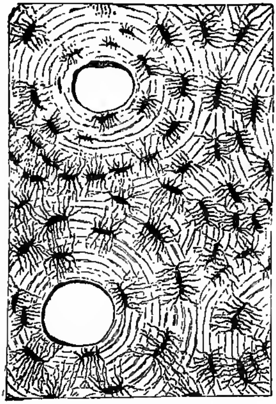

FIG. 175.—Human Collar Bone × 100
Transverse Section

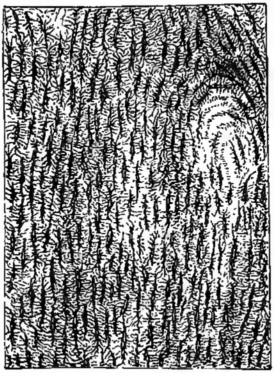

FIG. 176.—Fore-arm of a Turtle × 75
Transverse Section

posed to be cut across the Haversian canal) differs in the great classes of animals, so that a bit of bone will usually tell of itself whether it belonged to mammal, bird, or reptile. Thus, in the reptile bone shown in fig. 176, the lacunæ are longer but narrower than in the human bone: also the bone being a flatter one, they tend more to straight parallel rows. By these lacunæ and their caniculi, which are filled with protoplasmic matter, communication is kept up throughout the bone with the Haversian canals and larger hollows, and vital action maintained. It is remarkable that there should be a general correspondence in the size of the lacunæ, and those of the blood-corpuscles of the animal to whom the bone belongs: but the caniculi are far too small for blood-circulation.

**Skin and Hair.**—Passing now to the external surface of the body, the *skin* presents many points of interest. Transverse sections or layers will display the ridges and furrows as in fig. 177, and at intervals the mouths of the pores by which perspiration is exuded. These pores were counted by Dr. Erasmus Wilson, and found (on the palm of the hand) to number rather over 3,500 in a square inch. On an average the tiny tubes of which they are the mouths, penetrate to a depth of about a quarter of an inch, making a total length of drainage-tubes in that small area, of about 73 feet! It is easy to see the serious results which must follow if such an amount of drainage is not kept free and open; and what work must be thrown upon other excretory organs (such as nasal passages or kidneys) beyond their proper share.

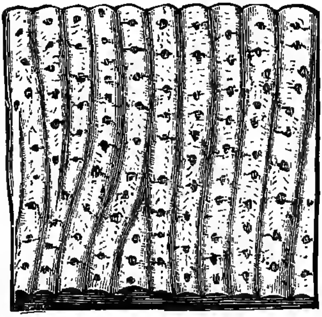

FIG. 177.—Surface of Skin × 75

Fig. 178 is a vertical section of skin. At *a* is the epidermis, or scurf skin, raised in ridges, the outer part of which consists of dead horny matter which is constantly peeling off, as can be seen very prominently in a section of the skin on the sole of the foot. The inner portion or Malphigian layer receives projections or *papillæ d* of the true skin beneath, which are supplied with capillary blood-vessels and nerve endings (see fig. 181). Deeper down in the true skin are the perspiratory glands, *e*, each gland a coiled up narrow tube, which finally passes out to the surface, assuming through the epidermis a cork-screw spiral form. The glands lie amongst masses of fat-cells *c*.

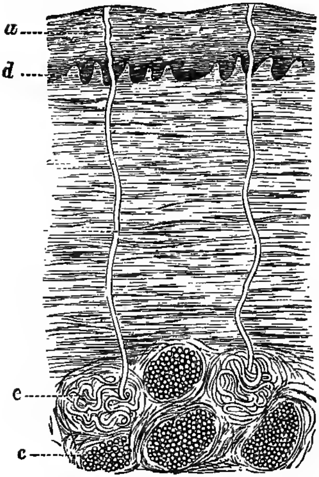

Fig. 178.—Vertical Section of Skin

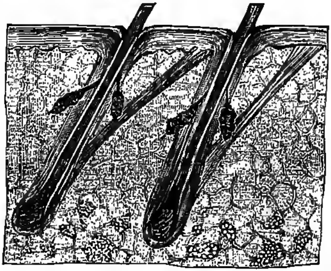

Fig. 179.—Scalp with Hairs

The skin is also furnished with sebaceous or oil-secreting glands, not very different in structure from the preceding. They are most conspicuous round the roots of hairs.

Fig. 179 is a section of the skin of the scalp, showing two hairs and hair-follicles. The sebaceous glands and muscles by which each hair can be moved, are clearly seen. A double-stained section of the scalp is a very beautiful object, as are double-stained preparations cut so as to give *transverse* sections through the hairs and hair-follicles.

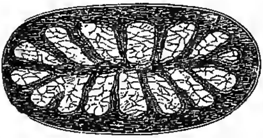

FIG. 180.—Hair of Peccary Transverse Section

Many hairs themselves are very interesting, and the most marked varieties are always to be purchased. The whiskers of lions and tigers give very large transverse sections. Fig. 180 shows the very peculiar arrangement of the medullary and cortical portions of the hair of the peccary. The hair of the sable is remarkable for the very large rounded cells occupying the centre. A most beautiful object is a good double-stained and 'injected' section of the *lip* of a cat or rabbit, showing the sensory hairs, and their follicles and appendages.

**Nerve Endings.**—This leads naturally to the organs of *sensation*, through which we are impressed by the external world. We will only mention here the sensations of touch, of taste, and of sight.

The skin itself is an organ of feeling, and fig. 181 is a much more magnified view of the *papillæ* seen in fig. 178, with the epidermis stripped away. Every one contains a loop of capillary blood-vessels, shown by the darkest shading. Many of them contain also the ends of minute nerves, shown

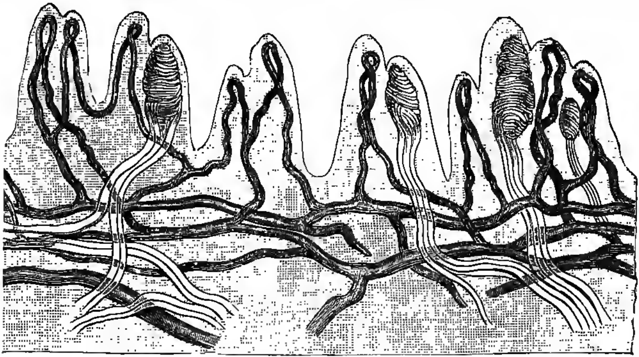

FIG. 181.—Papillæ of the Skin, showing capillary vessels and touch-bodies

in white, and which are twisted into oval bulbs. These are generally considered and termed 'touch-bodies.' It is by no means easy to find a section of skin which exhibits one or more of these touch-bodies really well, as they elude the section-knife owing to their softness and minute size.

The more delicate sensations, as we should expect, require more complicated mechanism. The terminal taste organs are chiefly found in a certain region of the tongue covered with large papillæ, called *circumvallate* papillæ, between which are pits. In the hare and rabbit these pits become more or less parallel leaves and grooves, more easily cut across, and the tongues of these animals are therefore usually sectioned to show the taste organs, which resemble minute necked flasks, whose mouths open into the sides of the pits or grooves (fig. 182). The flask-like bulbs are more clearly shown in fig. 183 under a higher power, and it will be seen that the outer part of the bulbs consist of oblong

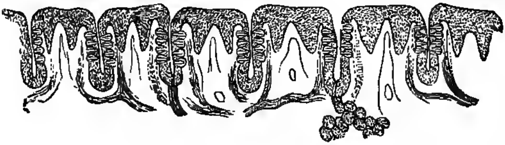

FIG. 182.—Taste-organs in tongue × 25

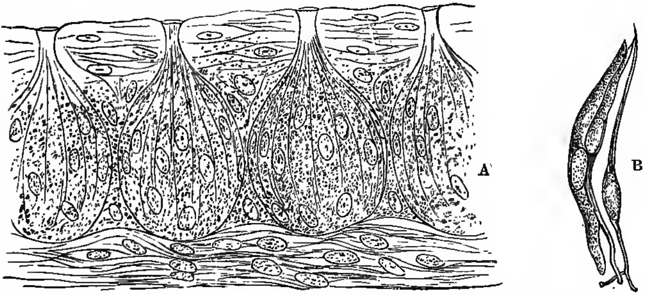

FIG. 183.—Taste-organs × 450. A. Flask-shaped bulbs B. Taste-cell with outer cells

cells, each containing a nucleus, and being tapered towards each end. Inside these are the true taste-cells (see right-hand cell in B) in which the central portion containing the oval nucleus is drawn out into an exceedingly fine process at each end. The outer end projects a little through the mouth of the flask like a tiny hair, the other is usually branched, and connected with the nerve-fibres below; but the precise mode of connection has even yet hardly been made out.

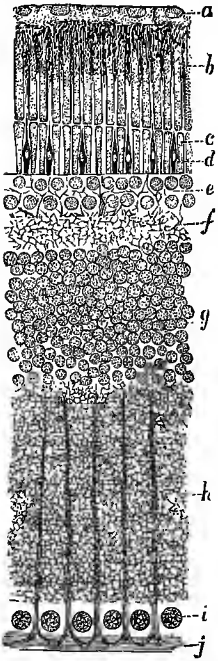

FIG. 184.—Section of Frog's Retina
*a.* Layer of pigment cells
*b, c, d.* Rods and cones
*e, g.* Nuclear cells
*f, h.* Molecular layers
*i.* Ganglion cells *j.* Nerves

Our last example is from the nerve-endings connected with vision. How an 'image' is thrown upon the retina at the back of the eye-ball, has broadly been shown in fig. 11; and it has been indicated that the rays which form this image are inconceivably minute and rapid *vibrations* in the ether, propagated along the course of the 'ray.' Organs to receive such delicate impressions must themselves be of almost inconceivable delicacy; and fig. 184 is a section of the retina only, from the eye of a frog. Here *j* are fibres from the optic nerve, and *i* large ganglion cells connected with them: these ganglion cells and the smaller nuclear cells presently mentioned appear to act in a manner as relays or fresh centres of nervous energy. At *h* and *f* are what are called molecular layers, and alternating with them at *e g* are two layers of nuclear cells, of which it may be remarked that while in the frog the layer at *g* is much thicker than at *e*, in the human retina the reverse is the case. The outer nuclear cells are connected by nerve-fibrils with a layer of extremely fine *rods and cones*, thicker at the base *c d* than the outer ends *b*. These inconceivably fine rods and cones (which are free or unconnected, whilst up to their bases the whole structure is joined by connective tissue) are received universally as the seat of vision. The remarkable thing is that in Vertebrates (owing to that outside-inside embryonic development indicated on p. 245) they are at the *back* of the retina, and the rays have first to pass through all the preceding layers, which are transparent. The analogy is pretty evident between these rods and cones and the *cilia* of certain epithelial cells.

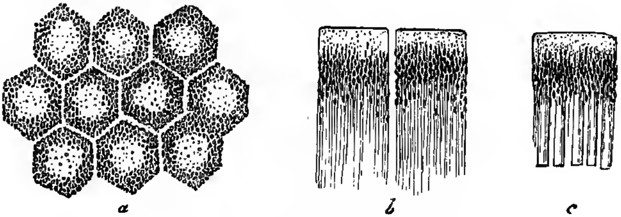

FIG. 185.—Pigment Cells of Retina

At the further end *a* of the layer of rods and cones is a sort of pavement of pigment cells, hexagonal in shape, as at *a* in fig. 185. The side next the rods throws out a number of pigmented fibrils (*b*), which pass down amongst the outer ends of the rods and cones as at *c*. Light causes these fibrils to stretch down farther; darkness to recede. They appear to have an important office. The outer ends of the rods and cones contain a colouring matter termed the visual purple, which *bleaches* on exposure to the light. But the colour returns when the rods and cones are in contact with the pigment cells, even in the dead retina, and probably also in the living one.

All these details of the intricate and beautiful mechanism are known; but *how it acts* is a profound mystery still.

Some think the fine rods and cones 'feel' the vibrations. Others rather believe that the bleaching of the purple, and its renewal by the pigment cells, are of the nature of a photographic process, and that *this* is vision. But no one knows. No one knows the action of the complicated layers of nuclear cells; no one knows the nature of the defect in the organ which makes some people colour-blind. Defects in the eye as an optical instrument, we can trace out to the last detail, and remedy with our prisms and lenses: the nature of the *vital* mechanism, no one has yet been able to understand.

The organs of smell and of hearing are equally wonderful in their minuteness of structure; but reference must be made for them to works upon anatomy or histology. Many other organs supply beautiful objects, especially when (as is often done) the blood-vessels are injected so as to show arteries red, and veins blue, the tissues also being stained. Sections of liver and kidney so treated are not only of great beauty in themselves, but will give a realistic sense of the exceeding delicacy of the mechanism of these important secreting organs.

The above will suffice most microscopists for a general collection: some others can only be just mentioned. The various glands and secreting structures of the stomach and intestines are both very interesting and very beautiful, affording some of the clearest and most exquisite examples of the cells of columnar epithelium. A fine illustration of the arrangement of tendons at a joint, is furnished by a longitudinal section of a 'decalcified' joint in the tail of a mouse. A developing tooth, or the entire jaw of a kitten or some other small animal, is a very beautiful and interesting slide. So is a section through the entire eye of a bird, or of a newt; or the entire head of a young newt or frog. This brief list is entirely confined to objects which are almost always to be obtained, most beautifully prepared by the professional mounter.

# CHAPTER XII

## MISCELLANEOUS MICROSCOPIC OBJECTS

In this chapter we collect a few notes on various subjects of microscopic study, or classes of objects, all of which are attractive, and some of which secure almost altogether the interest of specialists. We must be content with indications.

**Diatoms.**—One well-known authority has for some time never spent less than 100*l.* a year upon these beautiful objects, which have been termed 'Nature's jewels.' Many microscopists have studied little else for years; and there is no doubt that the attempt to make visible the finer of their markings, ranging in some cases to 100,000 per inch and upwards, has worked marvels in the improvement of microscope lenses. Hence it will be understood that anyone intending to take up this study seriously, *must* possess first-class objectives, both as regards aperture and definition. But the mere beauty and symmetry of scores of the larger forms can be seen with ordinarily good half and quarter-inch powers.

Diatoms are generally considered unicellar plants, growing in a silicious two-valved case,[1] and are found in all sorts of places. In fig. 186 are a group of fossil marine forms, some of which are still found on the surface of the sea. No. 1 is a *Surirella,* No. 2 a *Pinnularia,* No. 3 *Gomphonema,* No. 4 one of the genus *Navicula,* No. 5 a cross section or broken piece of *Melosira.*

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[1] Though it be heresy to say so, I am extremely doubtful of this. Many years since I was most unmercifully 'chaffed' by a small body of microscopists for presumed ignorance in the expressed belief that both *Volvox*, and at least some of the Diatoms, might probably be ultimately relegated to the animal kingdom. Some of the best recent biologists have already adopted that view as to *Volvox*: diatoms are not yet so regarded. But the character of the motion in some forms, and of the silicious skeleton in most (it is not clear to me that all so-called Diatoms are on the same plane of existence) are of great weight from analogy; while I think that in some cases fine pseudopodia can be observed in the living form. And some of the reasons which were once powerful in deciding for vegetable character—one for instance was the presence of chlorophyll—are admittedly now of no weight at all. I have however no title to speak with authority, and merely—for what I think adequate reason—put my doubts upon record.

Diatoms are also found attached to sea-weeds, as green or orange or reddish scum on the mud at the bottoms of ponds and brackish river-banks near the sea, and in all sorts of places. The beautiful *Arachnoidiscus* (fig. 187) is found in places as diverse as upon fronds of sea-weed, and stale jam! But the most ample supplies of many forms are fossil, in what are called diatomaceous earths, or in guano. The tooth-powder sold as 'Zozodont' will furnish for sixpence a large quantity, being a diatomaceous earth, but it varies a great deal; some year or two ago there were many fine forms in it, but the last sample I obtained contained only very common forms, resembling those in fig. 186.

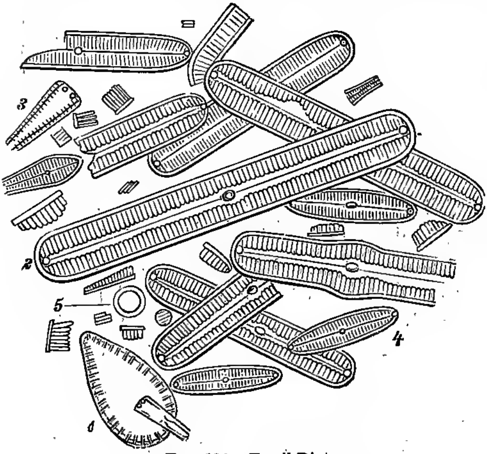

Fig. 186.—Fossil Diatoms

The most beautiful forms are the discoid or equi-angular ones. Fig. 187 reproduces the drawing of the late Mr. Richard Beck of the beautiful *Arachnoidiscus,* showing both of the two sides of the valve (which are different in all diatoms) and the way in which the two valves are joined into the 'double atom,' in this case resembling a pill-box, the whole

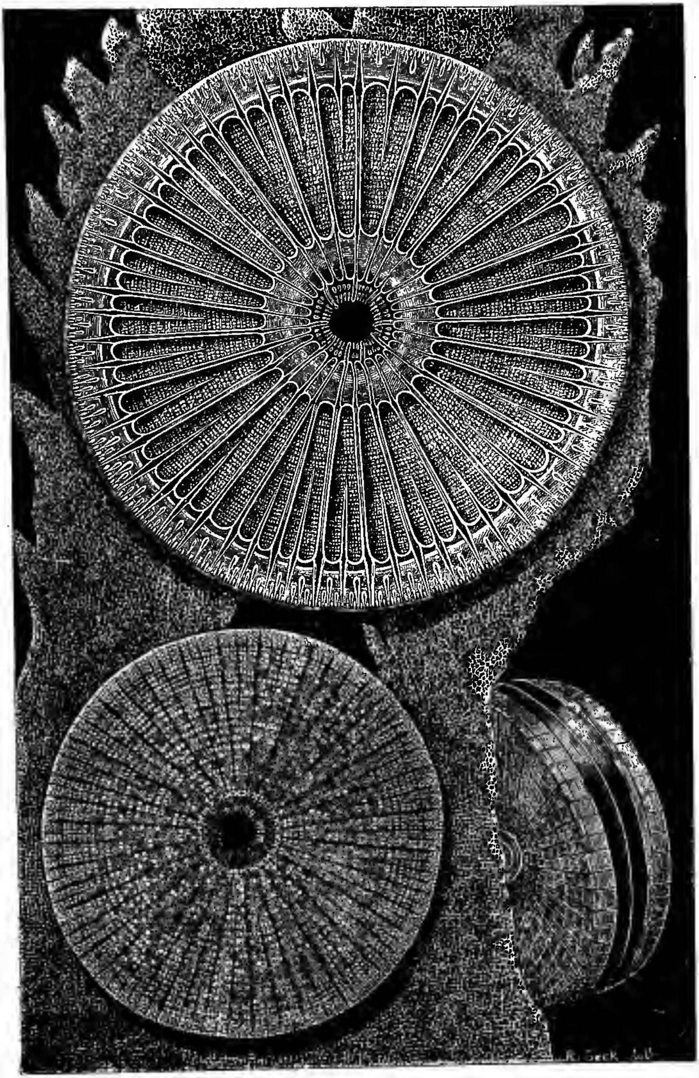

FIG. 187.—Arachnoidiscus Japonicus × 450

being seen edgeways. There are several varieties of *Arachnoidiscus*, with no really essential difference: one called *A. ornata* is perhaps the most beautiful of all. Fig. 188 is another circular Diatom called *Heliopelta.* There are varieties of this, with a different number of sections in the disk; but all are alike in the fact that the sections are not flat, but undulate; hence the whole valve cannot be in sharp focus at once. This is common to many discoid diatoms; while others resemble a convex shield. In such the more minute markings can only be focussed in a certain portion at a time.

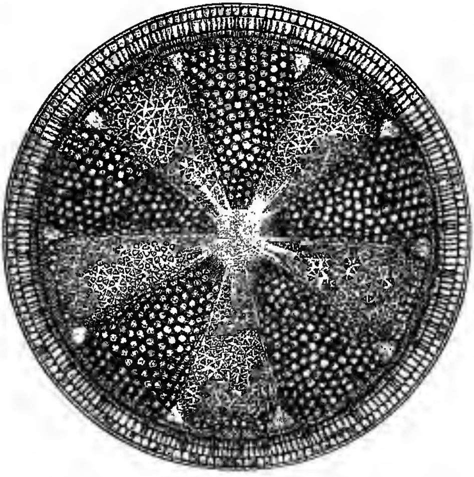

FIG. 188.—Heliopelta × 200
(*From photo by Mr. A. A. Carnell.*)

Fig. 189 is a larger representation of another *Navicula* (so named from resemblance to the deck or plan of a boat). There are many varieties of *N. lyra,* differing much in coarseness of marking, and one or two are now separated under other names, such as *N. maculata.* Some are much flatter towards the edges than others; and of every diatom selected, endeavour should be made to secure specimens which lie flat and focus as evenly as possible, either all over, or all round the edge, except when it is desired to show the edge or side view.

Of equi-angled diatoms, *Triceratium favus* is three-cornered, and similar marking but much coarser is found in

*T. septangulatum*, with seven corners. A much larger three-cornered diatom, still coarser in marking, and with rather bulging sides, is *Trinacrium*. In all these and many others, inside the larger marking alone apparent under moderate powers, are a number of much smaller markings of various kinds, some of which demand the very highest powers and skilful illumination to make them visible.

Thus, *Coscinodiscus asteromphalus* is a discoid diatom, which when ×100 appears the size of half a crown (for an average specimen: the size of many diatoms differs greatly) and the portion in focus (for it is not flat) appears studded with dots or spherules (according to focus and excellence of the objective) of which about 40 in line go to an inch; these spots therefore average about 4,000 per linear inch, and are the *larger* markings. But when × 2000 each 'large' circle is surrounded by a ring of smaller round spots, and within it are a number of much smaller spots. And a most minute difference in focussing, with the same illumination, changes the whole system from white to black, so that one image is like the photographic negative compared with a positive. This class of work, and many of the finer diatoms, require immersion lenses and high-class condensers.

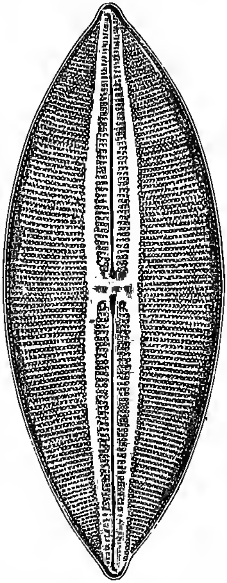

FIG. 189.—Navicula lyra ×600 (*Photo by Mr. C. L. Curties.*)

This difference in appearance from a mere shade of focussing, will show the exceeding difficulty of determining diatom structure. It appears as if the very fine dots were on, or apertures through, a very thin layer below or above a thicker one with larger apertures. And the general opinion is that the valves of diatoms are composed of either two or three layers. It may differ in various genera, and many good authorities believe that *P. angulatum* consists of apertures in a thicker layer, probably of silex, which is between two inconceivably thin ones, possibly of membrane, or a thin ectoderm. That the small spots are *either* perforations, or hollows, has been proved by the fact that Mr. C. H. Gill has succeeded in filling them with pigment. The further fact that when by proper arrangements we have got rid of the many spurious 'diffraction-images' caused by a small cone, which in times past have greatly obscured the subject, there remain with a wide *aplanatic* cone and good objective two opposite appearances and two only—a white spot or a black dot—also seems conclusive that each spot is *either* an actual aperture, or a section like a very small lens.

Under low powers many diatoms appear very beautiful, but instead of the marking, to be covered with glowing iridescent colour. This *is* iridescent colour, caused by interferences of the light passing through their regular marking as through a diffraction grating. A higher power, if of sufficient aperture, resolves the flat colour into dots or spherules.

*Living Diatoms.*—The motion of some of these is very interesting. The best for demonstration are *Bacillaria paradoxa*, of which a number of the rod-like forms adhere and *slide* along each other in a curious way: and *Pleurosigma quadratus*, resembling *P. angulatum*, but much shorter and broader. These move over the slide freely in a drop of water, in a very conspicuous way. Both are to be found pretty easily in the Thames estuary.

*Test Diatoms.*—For the purposes of this book, these have been sufficiently treated of in earlier pages.

**Foraminifera.**—These minute calcareous shells are found of many forms, which exhibit a wonderful variety. Many beds of chalk scores and hundreds of even feet thick are formed of them (p. 264), but hundreds of species are to be found in their detached form. ‘Spread’ slides containing many species from the ocean-bed in various localities are always to be obtained, one of the most attractive being the ‘Globigerina ooze’ brought up in the *Challenger* expedition from the bottom of the Atlantic. The Mediterranean also furnishes many beautiful forms, and so does the estuary of the Dee.

There are two sources of supply ready to the hand of every microscopist. The first is in new *sponges*. Everyone knows the sand that soaks out of a newly-purchased sponge. If this be carefully collected and examined, in a large proportion of cases (not of course always) this sand will be found to consist of Foraminifera. These may either be mounted on a black ground in a dry cell, as opaque objects, or spread upon a slip and mounted in balsam.

The other source is the sea-shore. With two or three little bags or bottles, and a spoon, the collector may walk along high-water mark, and low-water mark, and at each level scrape up a little of the cleanest and whitest-looking sand left *in the ripples* caused by retiring wavelets, in fairly calm weather. Stormy weather mixes things up too much, and the loose sand among the weed at high-water mark also fails for the same cause to yield other than small results. Between high and low water, a little of the sharp whiter sand should also be scraped up where tiny little pools are scooped by the water round the corners of rocks or stones. Of course the Steinheil *loup* or other pocket lens should be used from time to time, to see if there is anything to be got at each spot attempted. Any chance of dredging off shore in the ooze or mud or sand, is also not to be despised; and the blue clay-mud at the mouths of many estuaries will also yield specimens.

The produce of these proceedings has next to be washed and cleaned. First the salt and mud are washed away in several waters. Next the mass is to be passed through two sieves—the top one of perforated zinc or coarse gauze with meshes or holes about $\frac{1}{8}$ inch, which will take out weed and other large masses; the lower one of wire or silk gauze 120 to 150 meshes per inch. If dirty, boil in solution of potash, then again in water to dissolve the potash out. Finally dry entirely in the oven, and when cool throw into water and stir: the shells being full of air will mostly float on the surface and can be separated, while the solid sand will sink to the bottom. Further sorting out, into sizes or otherwise, can be carried out as desired.

Some of the Foraminifera exhibit well by dark-ground illumination: but as a rule they are best treated as either opaque or transparent objects.

**Radiolaria.**—The most beautiful of these minute forms of life, however, are the silicious or flinty shells of the group of animals named by Ehrenberg *Polycystina,* but which are since merged into the larger group known as *Radiolaria.* They are what it is the fashion to call 'single-celled' animals 'without organs'; nevertheless their leading characteristic is that a central portion is enclosed in a capsule of membrane, within which reproductive growth takes place, while the extra-capsular portion extends pseudopodia, and by these procures food. According as the membranous capsule is pierced by many fine pores, or by one or more larger apertures for communication, the Radiolaria are grouped into two great divisions of *Porulosa* or *Osculosa.* Further details of classification cannot be entered into here.[1]

The flinty skeletons of these animals, of which the term *Polycystina* is still applied to those in which the skeleton is outside the capsule, are as transparent as those of diatoms, and they consequently make splendid objects for dark-ground illumination. Fig. 190 represents a few shells thus shown, reproduced from a fine drawing by Dr. Dallinger. All these forms are very common in a thick bed of silicious sandstone found in Barbadoes, which almost entirely consists of such minute silicious shells. The present living forms appear to live chiefly on the surface of the sea, from which their shells sink after death, forming an 'ooze' at the bottom of the ocean. From this ooze the *Challenger* expedition has made known thousands of varieties. Such an ooze, if uplifted as dry land by geological change, would doubtless result in a silicious sandstone much resembling that of Barbadoes.

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[1] The classical work on the subject is Hæckel's magnificent Report on this department of the *Challenger* expedition.

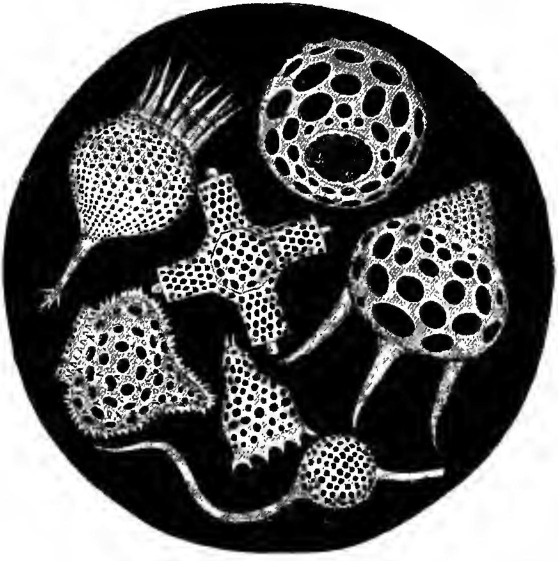

FIG. 190.—Polycystina × 100

On page 10 is shown a number of additional forms, all fossil, reproduced from a most beautiful set of drawings made from nature by the late Mr. Mungo Ponton. Many of the specimens figured are of exceeding rarity; and I have made selection either for this reason; or for unusual beauty; (omitting purposely those shown in the drawing by Dr. Dallinger), or lastly, to show by simple ocular demonstration what amazing complexity and minuteness of *real structure of some kind* must lie hidden in what it pleases biologists like Professor Haeckel to term a 'simple cell,' or 'a speck of primeval slime.' Gradual development is evident enough: for instance, gradual evolution seems clearly written on the forms along the bottom of the page. But what must there be in the 'single cell' which inhabits, *and has formed*, such a lovely dwelling as the central one of that bottom row; and if his *a priori* theory of Life leads a man to say there is no 'organisation' there, surely it must be said, So much the worse for the theory! It is certainly not the microscope's fault; it is that of the anti-teleologic, and therefore unphilosophic biologist at the eye-end of the instrument: the microscope itself has done its very best to show him his folly!

**Echinus Spines.**—Thin sections across these are very beautiful and perfectly symmetrical objects, which amongst the various genera and species afford a marvellous variety. In a lecture upon 'Star Fishes' by the late Dr. W. B. Carpenter, which I once had the pleasure of demonstrating for him with the projecting microscope, if my memory serves me aright he had nearly twenty specimens of these sections, besides his other preparations. A few generally find a place in every collection, and exhibit well either by transparent or dark-ground illumination.

**Mineral Sections.**—These are a distinct, large, and interesting class of objects, which furnish a study in themselves. Many of the forms require polarised light for their full understanding, and those who pursue this study seriously will need the aid of a properly fitted petrological microscope. But very much interesting detail does not require this. A very moderate power will suffice to show that most varieties of chalk are almost entirely composed of the compressed remains of Foraminifera (fig. 191), and that these and much larger organisms—largely consisting of Encrinites—compose the bulk of many carboniferous limestones (fig. 192). Other organic rocks, fossil Corals and Madrepores, Coal sections, and fossil Woods supply many beautiful objects. Inorganic minerals also frequently display very beautiful phenomena of colour and iridescence, but as a rule the polariscope is needed to bring out their beauty.

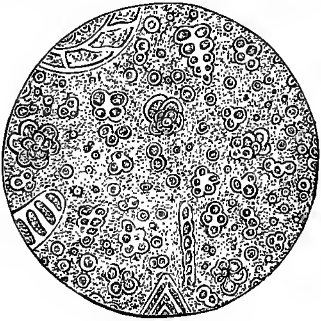

FIG. 191.—Chalk

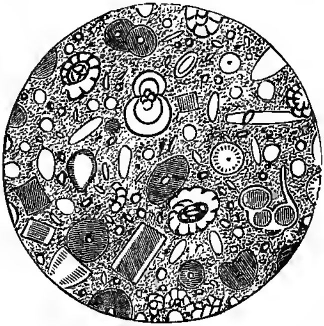

FIG. 192.—Limestone

**Polarising Objects.**—Any ordinary section of granite, for instance, is a beautiful object when seen by polarised light (fig. 193). By ordinary light its main constituents of mica, quartz, and felspar can be fairly made out; but when the polarising prisms are brought into action, their different qualities cause the most beautiful 'play' of colours. Some of them will give two colours only (always 'complementary' pairs of colours) when the analyser or polariser is adjusted in its two main positions: but other constituents have the property of exhibiting *every colour in succession* as one of the prisms is rotated. This last phenomenon depends upon the precise direction in which the section cuts a particular constituent part of the mineral; and occasionally some minerals—such as amethyst, some agates, chalcedony, and some opals, exhibit bands of these succession colours of enchanting beauty. Any dealer in this class of slides can select an assortment of any extent specially adapted for colour display.

Another class of colour phenomena is shown by sections of many crystals, when polarised light is used in the form of a *wide cone* sent through them by the condenser, the cone crossing in the crystal and being re-collected by a very similar condenser system as an objective. These figures are not images, however, but *interference fringes* produced by the crystal; and their explanation must be left to treatises upon Petrology or Physical Optics.

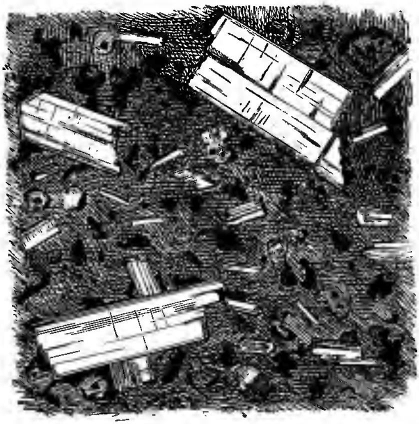

**FIG. 193.—Section of Granite by Polarised Light × 15**

*Crystal Films* of various salts in solution, allowed to crystallise on a glass slip, and mounted when dry, are most beautiful objects, and present most gorgeous colours in polarised light. An immense variety are sold at 1*s*. each, but the student can purchase the salts and prepare for himself if he prefers. It costs nearly as much, however, by the time he has selected a good one out of his various trials. A drop of saturated hot solution spread upon a warmed slip, will enable the *process of crystallisation* to be watched; but the easiest way to exhibit this pretty phenomenon is to purchase a few slides of the *fatty acids*, which are prepared for the purpose. These melt at a gentle heat over a lamp, taking care not to crack the glass or melt the ring of varnish; beautiful crystals, in colours, are seen to shoot out as the slide cools. The same slide will serve scores of times, or until some accident happens to it.

If a film of crystals does not happen to show colours alone, the selenite can be used with it; but the contrasts are better when the colours appear alone. This however depends upon the thickness required of any particular salt. Most crystals will mount in benzol-balsam; but some gradually dissolve in this, and have to be mounted in copal varnish or castor-oil. The beautiful *Hippuric acid* crystals require the last medium to preserve. The acid is dissolved in absolute alcohol gently warmed, and if a drop is dipped on to a clean warmed slide, it will show the most extraordinary variety of effects, by alternately cooling and warming, breathing on the slide, &c. Eno's fruit salt, tartaric acid, borax, and carbonate of soda, are common domestic articles that crystallise effectively from aqueous solutions; and will give different effects if hastened over a lamp.

The favourite and beautiful crystallisation of *salicine* is best prepared by flooding a slip with a saturated solution in warm water containing a little alcohol. This is then held in forceps over a lamp, keeping level, at a moderate heat till the fluid has evaporated. The heat is then increased, when the film *melts* in its own 'water of crystallisation,' and gradually the beautiful little rosettes start out, the effect being hastened, and varied *ad lib.*, by alternate breathing and cooling and heating in turn. Some salts, such as sulphate of copper, and sulphate of copper and magnesia mixed, melt and crystallise in the same way; and heat will modify the crystallisation patterns of tartaric acid and many other crystals.

*Organic sections* show impressively the power of polarised light in revealing structure. Their variety is countless; and many which exhibit sufficiently well in the ordinary way, come out with added beauty and detail when this additional power of analysis is brought into play. Sections of bone and teeth generally polarise, but without adding much: but sections of *horn, hoof*, and *cartilage* are exquisite objects, and develop the details with startling effect. Every variety of *fish scale* makes an effective polarising object, and is easily cleaned and mounted, either in jelly or balsam. Many hairs polarise strongly; and a bit of horsehair crinoline, or alpaca, cleaned of stiffening or other contamination, and mounted in balsam, makes a very pretty object.

Another class of objects, and one that lends itself to, and opens up another line of, systematic study, may be found in what are commonly called the 'palates' of various molluscs, but are more properly known as the 'odontophores.' The organ consists essentially of a cartilaginous strap, bearing a series of rows of hard detached teeth (fig. 194). A portion of the strap at the back is generally tubular, and needs to be split up in the preparation. The odontophore is usually curled round at the front of the mouth so as to return back again, and powerful muscles at the two back ends work the front part where it is curled round backwards and forwards, so as to produce a to-and-fro rasping action, which rasps away the substance to which it is applied, even the shells of other molluscs, which are often thus cut through.

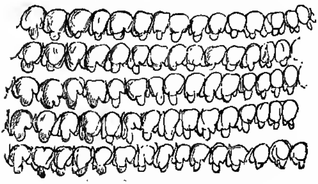
FIG. 194.—Five rows of teeth on Odontophore of Common Garden Snail

The teeth and their arrangement differ greatly in different molluscs, and a typical set is interesting. After dissecting out the organ it is simmered in hot solution of caustic potash, then well washed from soft integuments and dirty particles. Then it may be treated as other anatomical sections, mounting preferably in jelly for ordinary examination, but in balsam for polarised light.

Of course many vegetable structures also make beautiful objects. Amongst the most characteristic are the *starches*, every grain of which by polarised light exhibits a black cross, which *rotates* as the analyser or polariser is rotated.

But the space which can be allotted to enumeration of objects is exhausted. It will be readily understood that almost every division we have mentioned, is a world in itself, if followed up with the aid of any systematic treatise dealing with it: step by step the microscope will go hand in hand with the treatise, and give to it life, and a *reality* never appreciated before.

# CHAPTER XIII

## MICROSCOPICAL EXHIBITIONS

Anyone known to possess a fairly good microscope, is likely to be asked, sooner or later, to use his resources in the instruction and entertainment of others. Such a request is reasonable, and to comply with it may be a duty; but it very often proves to be anything but a pleasure. I have often observed much pains and trouble bestowed, only to be treated by those who had requested it with such contemptuous indifference as to justify positive refusal on any future similar occasion. A microscopist should guard against treatment of this kind, even for the sake of the *conversazione* or other occasion on which his assistance may be desired. If it *is* desired, all parties should unite in the endeavour to make it of value. Proper accommodation and facilities should be stipulated for, and some aid towards securing the interest of the company. Granted these essentials, however, either one, or still better several microscopes, are capable of yielding much interest.

**Precautions.**—It is better, as a rule, not to use high powers on such occasions, unless the company is to some extent a *skilled* one; and for the same reasons exceedingly difficult objects should be avoided. At a regular microscopical *soirée*, it is different: there, an exhibitor may find it worth while to spend an hour or more in adjusting some special object, which may attract interest for the entire evening amongst the many experts present. Even then, constant vigilance is necessary to prevent serious damage to valuable apparatus by rash handling from those not expert; but how much more in a mere general company. To meet this danger, several opticians actually supply 'dummy' milled-heads to go over those working the rack motion, so that the ignorant spectator in turning them may not be *able* to rack the objective down upon the slide. Such a fact proves how real the danger has been found to be. Moderate powers will be found sufficient, especially with good modern lenses and deeper eyepieces, to show well all that can be shown with profit in a general company.

**System.**—The chief necessity for giving real and living interest to a popular microscopic exhibition, is that it should take place upon some system, and that the idea of the exhibition should be generally known to the company. A regular microscopical *conversazione* is not here considered. At such it is usual to simply have a card describing each exhibit, which may remain for the whole evening, the company finding their interest in inspecting the exhibits at a hundred or more instruments in succession. We are here considering either a single instrument, or a few, up to say half a dozen; and we desire to give corresponding interest to these. Experience proves that this is not done by the common plan of just changing a slide now and then, and in an order the company knows nothing about, except from what they may happen to see upon haphazard inspection.

But now let us systematise the matter. If there be but one instrument, let it be understood that some *subject* will be illustrated during the evening, and that the slides illustrating it will be changed at set definite times, say from every five to every fifteen minutes. Subjects from the insect world, for instance, are very popular and suitable for such occasions. Going back to our chapter on that subject, either of the two methods there illustrated, or any other, might be adopted. At the stated fixed interval, another slide in the series will be substituted for the last; and the series may be either accompanied by one brief oral description of its main features, given loud enough for the room; or those features can be set out on a card, prepared for each slide beforehand. Everyone will *know* that at a certain time another item in the series will be ready for inspection, and the whole will have a connected bearing, and really add to intelligent knowledge.

With several instruments, the same plan may be pursued, in either of two ways. The whole team can be brought to bear upon *one* subject if preferred; and in that case the oral description, given audibly once for all, may be retained. Going again for example to the insect world, the various instruments may display a series of similar organs in various orders, or even genera; or upon the other plan, the prominent organs of one insect might be distributed amongst the whole. These might be then changed for another insect; and so on.

Or each several microscope may follow up during the evening its own individual succession of objects. In this case dependence must chiefly be placed upon cards, which should make clear both the nature of the series as a series, and the *points* of the objects from time to time brought forward to illustrate it.

Other departments, such as pond life, plant life, the anatomy and physiology of the human body, health and disease &c., lend themselves equally well to such systematic methods of exhibition and brief comment.

But though such is undoubtedly the most satisfactory method of microscopic exhibition, it may often happen that time, or knowledge, or opportunity, or material, may be insufficient to carry it out. In that case the microscopist must trust to the *attractiveness* of such detached subjects as he can exhibit; and it may be useful to indicate some such, which rarely fail to arouse more or less eager interest. When several instruments are in use, some of these may be worth exhibition during the whole time. Where this is not the case, change should still be made at *known intervals*, so that spectators may not be in ignorance whether or not they will find something fresh upon another peep.

**Exhibition Slides.**—A little expense is sometimes willingly borne, and a special class of very showy slides are prepared and well known as 'exhibition objects.' Such are symmetrical groups of diatoms, polycystina, spines and anchors, &c. Some 'spread' slides of the same are of equal beauty. Moderate powers, or dark field, such as will bring out iridescent colours in place of minute marking, will attract more admiration of 'Nature's jewels' than the fine detail of the diatomist as seen under very high power. Eggs of insects are treated in the same way, and some will excite much marvel. Groups representing sprays or bouquets of flowers are also prepared of iridescent butterfly scales, to be shown with the Lieberkühn. Such slides cost from 6*s*. to as much as 3*l*. Some of the groups of eggs, &c., show best in the same way.

**Live and Natural Objects.**—Mites of various kinds, as from sugar, cheese, &c., afford a great deal of mere amusement. The heads of living insects, secured as described (see fig. 22), are generally very attractive, as will be the head and jaws of a spider. If a flea can be procured and starved for a day or two to get transparent, and *gently* pressed (just to keep it still) in the live box, the beating of the heart or dorsal vessel will be very clearly seen.

An assortment of the smaller *flowers*, under low powers ranging from 4 inches to 1 inch, and illuminated by the bull's-eye, will often excite the liveliest admiration.

Circulation and cyclosis are always popular as described. The antenna of the large water-louse (*Asellus*) shows blood-movement well. A good inch objective will suffice for any of these.

Well-chosen specimens of *Pond Life* always attract attention. Rotifers, freed from dirty specks by several changes into *clean* water, and shown on dark ground, are very beautiful: their own instinct keeps them always in the focussed light of the condenser.[1]

**Plant Structure.**—In this department double-stained sections of stems; transverse sections of ovaries, buds, and flowers; various forms of pollen; and cuticles and sections of leaves showing *stomata*, are the most popular subjects.

**Miscellaneous.**—Sections of chalk and foraminiferal limestone, of coal showing organisms, and fossil wood are interesting. With the polariscope, sections of various horny tissues, and minerals, and series of crystallisations, are favourites: many crystallised salts are of marvellous beauty. The cornea of an insect's eye arranged to show multiple images of some brilliant object, such as a star in coloured glass, generally excites admiration. More need not be added for the class of exhibition here in view, as experience will readily suggest other subjects.

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[1] It may be of occasional service to many to state that Rotifers, Polyzoa, Volvox, and any other fresh-water microscopic life, can generally be obtained for 1*s.* per tube from Mr. John Hood, 50 Dallfield Walk, Dundee; or Mr. T. Bolton, Balsall Heath Road, Birmingham. Marine Polyzoa, Hydrozoa, &c., can be obtained alive (and often preserved, or mounted as slides) of Mr. J. Hornell, Biological Laboratory, Jersey (average 1*s.* 6*d.* per tube) or of the Director, Marine Biological Association, Citadel, Plymouth (which issues a price list). A very large and comprehensive catalogue of slides (the only one of the kind) in all branches of microscopy, published by Messrs. Watson & Sons. Holborn, will often be found of service.

**Lantern Exhibitions.**—A few sentences must suffice under this head. Insect subjects are peculiarly suitable for projection, and stand out clearly on the screen. In this case something in the way of a lecture will of course be planned. There might be a general review of the Insect World, giving types of organs in various insects; or a single class of organs may be taken, as the wings, or the mouth organs; or such a subject as Household Pests, or Household Insects, or Ponds and Ditches—such are actual subjects which I have myself demonstrated, either in personal lectures or for friends. Minerals demonstrate admirably by polarised light. Botanical subjects exhibit easily and well, and good double-stained sections are very beautiful. Sections of organs and parts of the human body exhibit well upon the screen up to about 2,000 diameters with the oxy-hydrogen light, which is sufficient for nearly anything a popular lecturer would desire. In illustrating for a medical friend a popular lecture upon 'The Skin,' I was able to show every point he desired, embracing in all about thirty slides. These brief hints will suffice to indicate what is within the reach of a really good projection instrument and competent demonstrator; and it only need be added, that the Lieberkühn and spot-lens are, for some objects to be shown upon a screen, very valuable and effective adjuncts.

# CHAPTER XIV

## ILLUMINATION AND DARK GROUND METHODS

Resolution is affected very greatly according to the particular wave length of the light employed. This will be understood if we remember that the light waves giving rise to red light are much bigger than those giving rise to green or blue light. Now we have in the microscope to combine these waves to form our image, and it can be seen that if our object, or details in such an object, are smaller than the waves themselves we cannot get correct images. Thus we find that light from the red end of the spectrum possesses little resolving power, whilst the further we can go towards the violet the greater amount of detail can we see.

Nor does the matter stop here. It is well known that beyond the visible violet spectrum there exists a further spectrum, called the ultra-violet, and this ultra-violet region is made up of exceedingly short waves; if structure, therefore, be examined by ultra-violet illumination still greater detail will be seen. Unfortunately, the ultra-violet waves are quite invisible to the eye, and to obtain pictures of structure illuminated in this manner we have to fall back on photography.

Apart from this drawback, special quartz slides and cover glasses are necessary, and the condenser, ocular, and objectives used must also be made of fused quartz, as the ultra-violet rays are stopped by glass. The process may prove of immense value, however, and its use and development in this country are almost solely due to Mr. J. E. Barnard, late president of the Royal Microscopical Society, who has already obtained some very suggestive results. It will not take the place of the ordinary methods, but it will give us another set of pictures of living material, which is what is wanted.

It will be understood, therefore, that the nearer we can work towards the violet end of the spectrum the greater amount of resolution will be obtained. For example, if a diatom be resolved using blue light, and the light be then changed to red, it will be found that resolution will fail. The ideal light will be a monochromatic one, approaching the green or blue end of the spectrum. In addition to obtaining greater powers of resolution, monochromatic light will improve the performance of all lenses of the semi-apochromatic variety immensely. Indeed, using green light it is almost impossible to distinguish, say, the Watson 4 mm. Holoscopic objective and the Zeiss 4 mm. apochromatic.

For most work strictly monochromatic light is not necessary and with stained histological specimens is often objectionable, as it of course materially alters the colour picture obtained. Coloured disks of glass can be obtained from all opticians. These fit into the condenser carrier and will be found of very great utility. The light blue, Spitta's pot green, and the yellow, perhaps, being the most generally useful. Messrs. Wratten produce an excellent series of screens which are of great utility. With the mercury vapour lamp, true monochromatic light can easily be obtained by means of suitable screens, and this is the best type of illuminant yet devised. Where the worker is using either the ordinary oil microscope lamp, or the electric lamp already mentioned, it will be found advantageous to use a light blue filter in the condenser carrier. This removes much of the yellow of the light and makes a very soft and agreeable illuminant to work with. Of course daylight is not suitable for critical work, though it will suffice for all ordinary low-power work with objectives up to, say, the ½ inch, and will be found useful for much histological work. From what has been said it will be understood that a light of great intensity is an advantage. Where it is found that the intensity of the light is too great for a given object, we should endeavour to reduce the intensity by a screen and not, if possible, cut down the cone by means of the iris diaphragm. It should be borne in mind, that when we use frosted bulbs, or place ground glass in front of our light source, we are altering the phase relation of the light very considerably. The ideal light is undoubtedly the Mercury vapour lamp; unfortunately, its price is high, being some £10, but for those who can afford it, there is no more useful light. Its dominant wave length is very small, and monochromatic light can be got from it very easily by means of proper screens. The arc-lamp is in most cases not suitable, upsetting the corrections of most objectives, especially for dark ground work. The Pointolight is another excellent form, and is very useful for bacteriological routine work or for micro-photography.

Where objects are too transparent for such intense illumination, it is far better to reduce the intensity of our light by means of screens than to cut down the cone from the condenser by means of the iris diaphragm. Such screens are sold by Messrs. Beck, and are ideal; indeed, with many objects full cones of light may be employed if such screens are used to reduce the light intensity. Above all, do not alter the dominant wave length toward the red, as has been at times suggested. If we are using an electric lamp, and, in order to reduce its intensity, give the light a redder tint, we lose in resolution, and make matters worse and not better.

**Dark Ground Methods.**—Examination by means of the dark ground is daily becoming more important. For all elementary work a suitable stop at the back of the condenser will suffice, good dark ground being obtained with objectives up to n.a. 0·65. The ideal lens for this method is the 8 mm. apochromat, which may be used with a ×20 eyepiece. Such dark grounds were at one time on the market, but have now been withdrawn. It is quite easy, however, for the student to arrange a suitable stop for himself.

For ideal dark ground illumination, however, we must use an objective with a higher n.a. than 0·65, which interferes with resolution. Probably the ideal lens for the majority of such work is the $\frac{1}{7}$-inch oil immersion of n.a. 0·95 made by either Messrs. Watson, Zeiss, or Leitz. To use this properly we must employ one of the special dark ground illuminators, which will be considered immediately. There is no doubt that far better results can be obtained with such a lens than with an ordinary $\frac{1}{12}$-inch oil immersion of, say, n.a. 1·30 stopped down by a funnel stop. These stops, as a rule, are unsatisfactory, as the stopping down should undoubtedly be done in the lens combination itself.

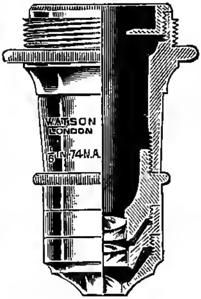

Fig. 195

There are two chief types of dark ground illuminators on the market—the reflecting pattern such as those of Leitz, Baker, and Swift, and the paraboloid type such as those made by Watson, Beck, and Zeiss. There is absolutely no doubt that the best type is the reflecting pattern, as has been demonstrated by Mr. Barnard, and, in my opinion, the paraboloid should be avoided. The best form at present on the market is, I think, that of Leitz, with which the most exquisite effects can be obtained.

The trouble with all these special illuminators is that they are not easily used unless the student will master the technique. Many dark-ground illuminators are condemned as useless, when the whole fault lies with the manipulator. Let us consider the method of using them satisfactorily. First ascertain that the mirror and lamp are in a line, and then place the dark-ground illuminator in position in the substage. In most of these illuminators a circle is engraved to enable centration to be performed Let us then place a low power, *e.g.* $\frac{2}{3}$-inch, objective on the nose piece of the microscope, and by means of the centring screws accurately centre the rings.

The object is now placed on the stage, after placing immersion oil on the illuminator. The oil should be thick, and sufficient in quantity to enable oil contact to be secured. No bubbles should be present. The lens to be used is now rotated into position and cautiously focussed, when some bright particles will be seen. The illuminator should then be gently racked up or down a little till the best illumination is obtained. At this stage ascertain that the centration is correct. This may be done by removing the ocular and looking down the microscope tube. The back lens of the objective should show as a black circle surrounded by a ring of light, which should be equal all round. If not, centration must be performed by the centring screws. The source of light should be a brilliant one, and it is best to secure a parallel beam by means of the bull's-eye condenser. The ideal illuminant is the mercury vapour lamp. The arc light should not be used.

An important point is that the film under examination should be in as thin a layer as possible, and air-bubbles must be carefully avoided. The objective must be less than n.a. 1, otherwise dark ground will not be obtained. Where a dark-ground illuminator has no circles engraved on the top for centration, it is best to make a small spot of ink in its centre, and use this as the circle to perform centration by. Where circles are engraved be careful, when the illuminator is finished with, to thoroughly clean off all oil with xylol, as otherwise the circles will become invisible.

Messrs. Beck have recently introduced an adjustable dark-ground illuminator that will give dark ground with an oil immersion objective of n.a. 1·20. The effects yielded are most beautiful, far higher resolution being obtained.

For the convenience of the student we append the full directions for the use of dark-ground illuminators, such as are supplied by Messrs. Leitz; if they are carefully followed success will be assured.

(1) **Directions for using Leitz Dark-ground Condenser.**—Fit condenser into substage sleeve in place of the usual Abbe, and see that the condenser, when racked up, comes exactly flush with upper surface of microscope stage.

(2) Use microscope in vertical position to avoid losing oil contacts.

(3) **Illuminant.**—A fairly powerful and concentrated form of illuminant is advisable, although with care the edge of flame of ordinary oil lamp will give good results. Arrange a bull's-eye condenser, to project either a parallel beam or focus an image of the source of illumination on to the microscope mirror. With a large or comparatively weak illuminant the latter course will generally be found preferable.

(4) Direct the light by plane mirror through condenser and focus upper surface of condenser with a low-power objective (not exceeding $\frac{2}{3}$ inch) and eyepiece about ×6, when two concentric rings engraved on surface will be faintly seen. (After previous use the condenser should be thoroughly cleaned and all oil removed, otherwise the ring will be difficult to observe.) By means of centring screws on mount of condenser direct these rings into centre of field.

(5) Take specimen to be examined, which should be on a slide of a thickness from 0·9 to 1·1 mm., and apply a little cedar-wood oil to under-surface and place on stage of microscope, then gently rack up condenser so that there is an oil contact between condenser and slide, care being taken to avoid air-bubbles.

(6) Focus specimen with low-power objective, and a bright spot of light will be observed near centre of field. This spot should be made as small as possible by raising or

lowering condenser. If instead of a bright spot a dark patch is observed, this points to the slide being too thick, and satisfactory results cannot be obtained. Next direct this spot of light into the centre of field by means of microscope mirror.

(7) The $\frac{1}{12}$-inch oil immersion objective, which must be fitted with a funnel stop to reduce its aperture to 0·85, should now be used in place of the $\frac{2}{3}$-inch, and focussed in the usual manner with oil contact with slide, and beyond, perhaps, a slight adjustment (to compensate for de-centrality of nosepiece, if any) no further adjustment other than choice of eyepiece should be necessary.

(8) Comparatively high-power eyepieces such as ×10 and ×12 or ×15 are the most satisfactory, although the choice is controlled by the power of illuminant.

(9) It is advisable to use thickened cedar-wood oil.

A dry form of dark ground illuminator was sold some years ago under the name of the Luft dark ground microscope. It proved very useful for much routine work and had the great advantage of being fool-proof. Essentially this form consisted of a dry achromatic condenser with a permanent stop in it. This stop was calculated for an 8 mm. apochromat which was used with a ×27 ocular. At my suggestion Messrs. Watson have made this form recently with very successful results.

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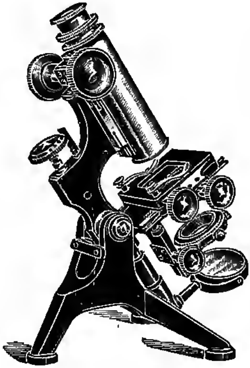

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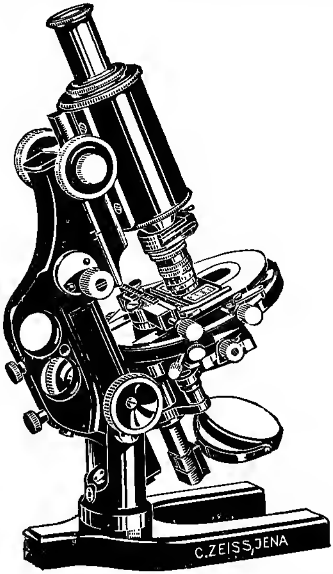

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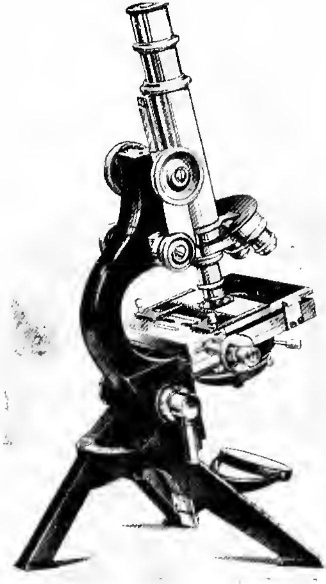

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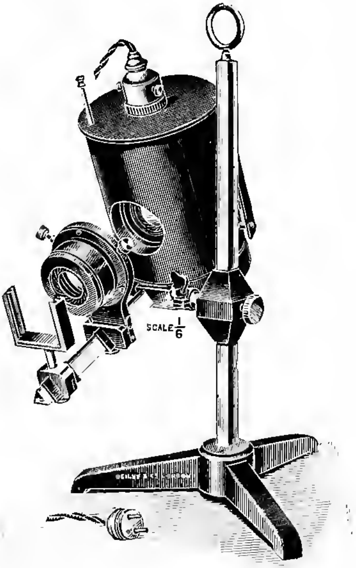

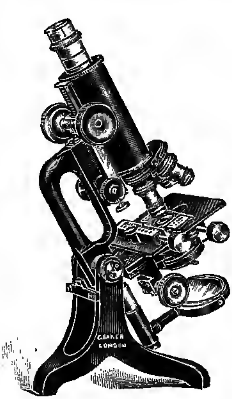

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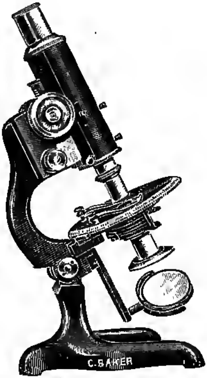

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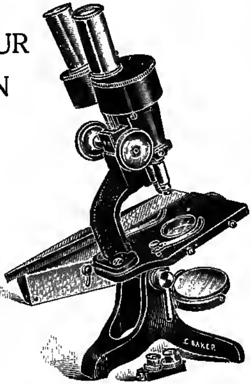

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